DESIGN SYNTHESIS CHARACTERIZATION AND BIOLOGICAL EVALUATION OF SOME NOVEL BENZOTHIAZOLE DERIVATIVES AS ANTI TUBERCULAR AGENTS TARGETING GLUTAMINE SYNTHETASE 1

A Dissertation submitted to
THE TAMILNADU Dr.M.G.R. MEDICAL UNIVERSITY
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in partial fulfillment of the requirements for the award of the Degree of

MASTER OF PHARMACY
IN
PHARMACEUTICAL CHEMISTRY

Submitted by
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CERTIFICATE

This is to certify that the dissertation entitled **"DESIGN, SYNTHESIS, CHARACTERISATION AND BIOLOGICAL EVALUATION OF SOME NOVEL BENZOTHIAZOLE DERIVATIVES AS ANTI-TUBERCULAR AGENTS TARGETING GLUTAMINE SYNTHETASE 1"** submitted by the candidate bearing the Register No:**261515701** in partial fulfillment of the requirements for the award of degree of **MASTER OF PHARMACY in PHARMACEUTICAL CHEMISTRY** by the TamilNadu Dr. M.G.R Medical University is a bonafide work done by her during the academic year 2016-2017 in the Department of Pharmaceutical Chemistry, College of Pharmacy, Madras Medical College, Chennai- 600 003.

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CONTENTS

LIST OF ABBREVIATION

ADME	Absorption, Distribution, Metabolism, Excretion
AIDS	Acquired Immuno Deficiency Syndrome
ART	Anti Retroviral Therapy
BCG	Bacilli Calmette Guerin
CADD	Computer Aided Drug Design
DOTS	Directly Observed Treatment Short –Course
GC-MS	Gas Chromatography And Mass Spectroscopy
GS	Glutamine Synthetase
HIV	Human Immuno Deficiency Disease
IR	Infrared Spectrum
LBDD	Ligand Based Drug Design
LC-MS	Liquid Chromatography And Mass Spectroscopy
Log P	Partition Coefficient
MABA	Micro Plate Alamar Blue Assay
MDR-TB	Multi Drug Resistant –TB
MIC	Minimum Inhibitory Concentration
NMR	Nuclear Magnetic Resonance
NRA	Nitrate Reductase Assay

OSIRIS	Optical Spectroscopic And Infrared Remote Image System
PDB	Protein Data Bank
PSA	Polar Surface Area
QSAR	Quantitative Structure Activity Relationship
REMA	Resazurin Microplate Assay
SBDD	Structure Based Drug Design
TB	Tubercle Bacillus
TLC	Thin Layer Chromatography
TPSA	Total Polar Surface Area
WHO	World Health Organization
XDR- TB	Extensively Drug Resistant-TB
µg	Microgram
ml	Milliliter
mg	Milligrams
˚C	Celsius
3D	Three Dimensional
mins	Minutes
hrs	Hours
%	Percentage

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INTRODUCTION

The most recent World Health Organization (WHO) reports show that there were 9.0 million new tuberculosis (TB) cases and 1.5 million tuberculosis (TB) deaths. Tb is the leading cause of death from an infectious disease worldwide, next to the human immunodeficiency virus (HIV). Co-infection with the HIV fuels the global TB crisis, and successful TB treatment is further complicated and hampered by the existence of multidrug- resistant (MDR) TB and extensively drug resistant (XDR) TB[1].

TUBERCULOSIS is a communicable disease caused by *Mycobacterium tuberculosis* (MTB), a disease that remains as one of the most alarming health problems worldwide. It is simply spreadable by sneezing, cough and speaking. Predisposing factors for TB include close contact with large populations of people. Humans are the only reservoir for the *Mycobacterium tuberculosis.*

Bedaquiline received U.S. Food and Drug Administration (FDA) approval in late 2011. The safety and effectiveness of these new agents are still uncertain, because they are based on the results of relatively small studies [2].

HISTORY :

Although Dr. Richard Morton established the pulmonary form associated with tubercles as pathology in 1689 due to the variety of its symptoms, TB was not identified as a single disease until the 1820s[3]. It was not named "tuberculosis" until 1839, by J. L. Schönlein[4] . The bacillus causing tuberculosis, M. tuberculosis, was identified and described on 24 March 1882 by Robert Koch[8].

Albert Calmette and Camille Guérin achieved the first genuine success in immunization against tuberculosis in 1906, using attenuated bovine-strain tuberculosis. It was called bacille Calmette–Guérin (BCG). The BCG vaccine was first used on humans in 1921 in France, but received widespread acceptance in the US, Great Britain, and Germany only after World War II[8].

TYPES OF TUBERCULOSIS :

Tuberculosis is a contagious disease; it affects almost all the important organs of the body. Clinically, tuberculosis is broadly categorized into three major categories

1. **Primary tuberculosis**: affects a person who had never been exposed to the bacterium[5]
2. **Secondary tuberculosis**: the bacterium regains its active mode and causes the symptom [5]. Secondary TB increases the chance of the infections spread to other organs such as kidneys, heart, and brain.
3. **Disseminated tuberculosis**: means that the tuberculosis has infected the entire body system. It primarily affects the bones of spines, hips, joints and knees and even the central nervous system. It infects the CSF, the GIT, the adrenal gland, skin of the neck and even the heart [6].

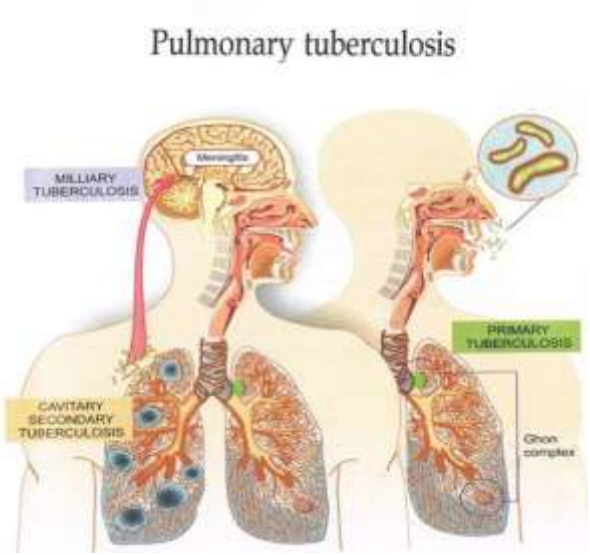


Figure 1 : Types of tuberculosis

CELL WALL

The well-developed cell wall contains a considerable amount of a fatty acid, mycolic acid, covalent by attached to the underlying peptidoglycan bound polysaccharide arabinogalactan, providing an extraordinary lipid barrier. This

barrier is responsible for many of the medically challenging physiological characteristics of tuberculosis. The composition and quality of the cell wall components affect the bacteria's virulence and growth rate.

The **peptidoglycan** polymer confers cell wall rigidity and just external to the bacterial cell membrane, another contributor to the permeability barrier of mycobacteria.

Another important component of the cell wall is **lipoarabinomannan**, a carbohydrate structural antigen on the outside of the organism that is immunogenic and facilitates the survival of mycobacteria within macrophages.

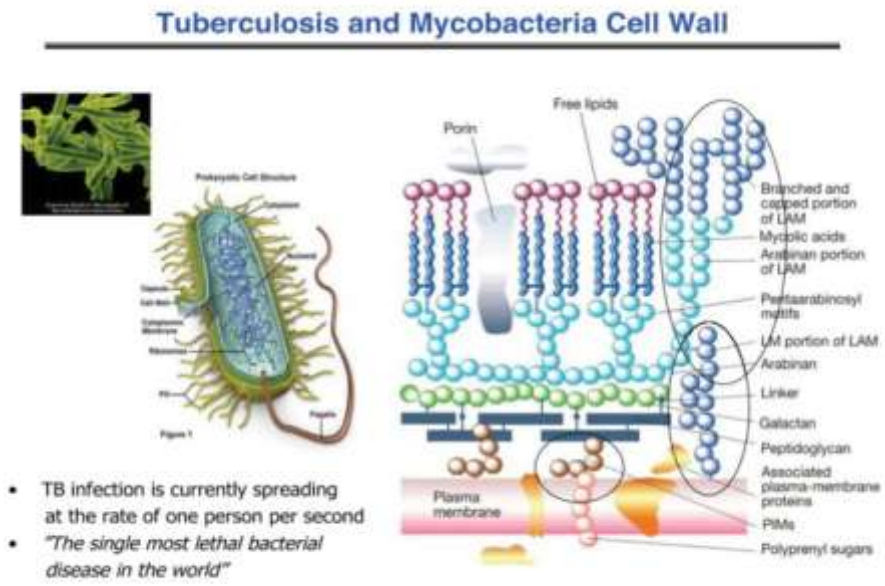


Figure 2 : Mycobacterial cell wall

The cell wall is key to the survival of mycobacteria, and a more complete understanding of the biosynthetic pathways and gene functions and the development of antibiotics to prevent formation of the cell wall are areas of great interest [8].

MECHANISM:

The followings are the stages in TB [6-8].

Stage 1: Droplets of nuclei are generated by during talking, coughing and sneezing. The droplets are inhaled. One droplet of nuclei contains more than 3 bacilli. The infection begins 7-21 days.

Stage 2: In the lungs, M. tuberculosis is taken up by alveolar macrophages, but they are unable to digest the bacterium. Its cell wall prevents the fusion of the phagosomes with a lysosome. MTB multiplies virtually unrestricted within unactivated macrophages until the macrophages burst.

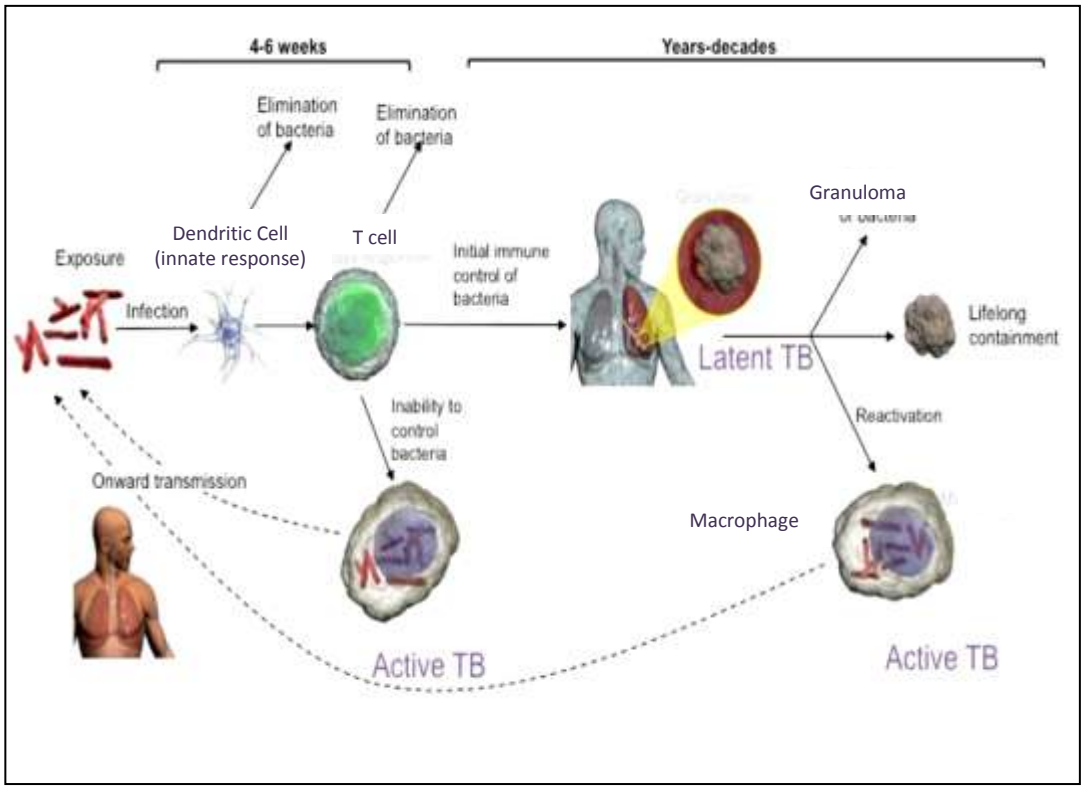


Figure 3 : Pathogenesis of Tuberculosis

Stage 3: The next stage, lymphocytes begin to infiltrate. T- Cells recognize processed and presented MTB antigen in context of Major Histocompatibility Complex molecules. This results in T- cell activation and the liberation of cytokines including gamma interferon (INF).

The liberation of INF causes in the activation of macrophages. These activated macrophages are capable of destroying MTB. At this stage, the individual becomes tuberculin- positive.

Stage 4: Many activated macrophages can be found surrounding the tubercles. MTB uses these macrophages to replicate, and hence the tubercle grows.

The growing tubercle may invade a bronchus. If this happens, MTB infection can spread to other parts of the lung. Similarly, the tubercle may invade an artery or other blood supply line. The hematogenous spread of MTB result in extrapulmonary tuberculosis otherwise known as military tuberculosis [8].

DIAGNOSIS[9,10] :

Diagnosing active tuberculosis based merely on signs and symptoms is difficult, as is diagnosing the disease in those who are immunosuppressed. A diagnosis of TB should, however, be considered in those with signs of lung disease or constitutional symptoms lasting longer than two weeks [14].

- A chest X-ray and multiple sputum cultures for acid-fast bacilli are typically part of the initial evaluation.
- Laic acid amplification tests and adenosine deaminase testing may allow rapid diagnosis of TB. These tests are not routinely recommended.
- **Mantoux tuberculin skin test** is often used to screen people at high risk for TB. Those who have been previously immunized may have a false-positive test result.
- Interferon gamma release assays (IGRAs), on a blood sample, are recommended in those who are positive to the Mantoux test. These are not affected by immunization or most environmental mycobacteria, so they generate fewer false-positive results.

PREVENTION :

Tuberculosis prevention and control efforts primarily rely on the vaccination of infants and the detection and appropriate treatment of active cases. The World Health Organization has achieved some success with improved treatment regimens, and a small decrease in case numbers.

TREATMENT[11,12]:

Vaccines :

The only available vaccine as of 2011 is Bacillus Calmette-Guérin (BCG). In children it decreases the risk of getting the infection by 20% and the risk of infection turning into disease by nearly 60%. It is the most widely used vaccine worldwide, with more than 90% of all children being vaccinated. The immunity it induces decreases after about ten years. A number of new vaccines are currently in development [15].

Drugs in used TB treatment[11] :

First line drugs: Isoniazid (H), Rifampin(R), Pyrazinamide (Z), Ethambutol (E), Streptomycin(S).

Second line drugs: Thiacetazone, Paraaminosalicylicacid, Ethinonamide, Cycloserine, Kanamycin, Amikacin, Capreomycin.

Table 1: World Health Organization recommended treatment regimens[14]

Intensive phase		Continuation phase	
Drug	**Duration months**	**Drug**	**Duration months**
Ethambutol# Isoniazid Pyrazinamide Rifampicin	2	Isoniazid¶ Rifampicin¶	4
Ethambutol# Isoniazid Pyrazinamide Rifampicin	2	Ethambutol¶ Isoniazid¶ Rifampicin¶	4*

#: ethambutol has to be prescribed in individuals with noncavitary, sputum smear-negative pulmonary tuberculosis (TB) or with extrapulmonary TB who are known to be HIV negative; in TB meningitis, it should be replaced by streptomycin. ¶: the World Health Organization considers the three times per week continuation phase as an acceptable alternative for any new TB patient receiving directly observed therapy.

EPIDEMIOLOGY[16]:

In 2015, 6.1 million new TB cases were notified to national authorities and reported to WHO. Notified TB cases increased from 2013–2015, mostly due to a 34% increase in notifications in India. In 2015, there were an estimated 10.4 million new (incident) TB cases worldwide, of which 5.9 million (56%) were among men, 3.5 million (34%) among women and1.0 million (10%) among children. People living with HIV accounted for 1.2 million (11%) of all new TB cases.

The best estimate is that there were 1.4 million TB deaths In 2015, and an additional 0.4 million deaths resulting from TB disease among HIV-positive people. In terms of cases, the best estimates for 2015 are that there were 10.4 million new TB cases (including 1.2 million among HIV-positive people), of which 5.9 million were among men, 3.5 million among women and 1.0 million among children. Overall, 90% of cases were adults and 10% children, and the male: female ratio was 1.6:1.

In the African Region where the burden of HIV associated TB is highest, 81% of notified TB, patients had a documented HIV test result. The proportion of known HIV-positive TB patients on ART was above 90% in India, Kenya, Malawi, Mozambique, Namibia and Swaziland.

NEED TO FOCUS ON TUBERCULOSIS DISEASE

- Tuberculosis is a leading cause of death worldwide. The World Health Organization estimates that one-third of the world's population is currently infected with *M.tuberculosis*, and that 1.3 million deaths result from these infections each year.

- In the year 2000-2016, there were around 10 to 15 million people with latent TB in the U.S & in 2007, 2.4 million cases were reported.

- In the year 2015, approximately 560 thousand new cases per year and 740 thousand new patients were infected by both MTB and HIV.

NEED FOR NOVEL ANTI-TUBERCULOSIS AGENTS

- A number of once active anti-tuberculosis drugs have now became inactive due to the ever-increasing rise in drug resistant strains of tuberculosis.

- The lack in patient compliance is because the current treatment regimen lasts 6-9 months. The length of the treatment period makes difficulty in killing off the latent and slow-growing bacteria.

- The multi -drug resistant TB (MDR-TB) occurs only when drug-susceptible TB is improperly or incompletely treated. According to the World Health Organization (WHO), MDR-TB is defined as a resistance to two of the most effective first line TB agents: Rifampicin and isoniazid[15].

- When a strain of TB becomes resistance to any fluoroquinolone and at least one of the three second-line agents (Capreomycin, Kanamycin and Amikacin), it becomes described at extensively drug-resistant TB(XDR-TB)

- Emergence of Multi-resistant (MDR) strains and high susceptibility of human immunodeficiency virus (HIV) infected persons to the disease forced the scientist to develop novel anti-tuberculosis agents[18].

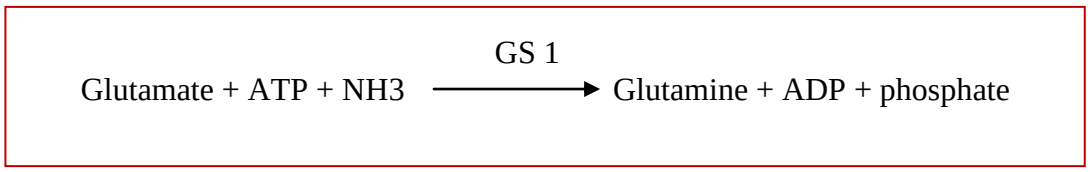
It is evident from these facts there is an ever-growing need to develop novel agents for the treatment of tuberculosis. These new agents should be potent, fast acting, have an excellent pharmacodynamics / pharmacokinetics (PK/PD) profile, have high therapeutic index, and preferably have a novel mechanism of action as to avoid cross resistance with other agents.

ENZYME PROFILE

Glutamine Synthetase 1

Glutamine synthetase is an enzyme that plays an essential role in the metabolism of nitrogen by catalyzing the condensation of glutamate and ammonia to form glutamine [18].

Glutamine synthetase (GS; EC 6.3.1.2, also known as γ-glutamyl: ammonia Ligase) has three metal ions in the active site between the two pockets, which are necessary for stability and catalytic activity[22].



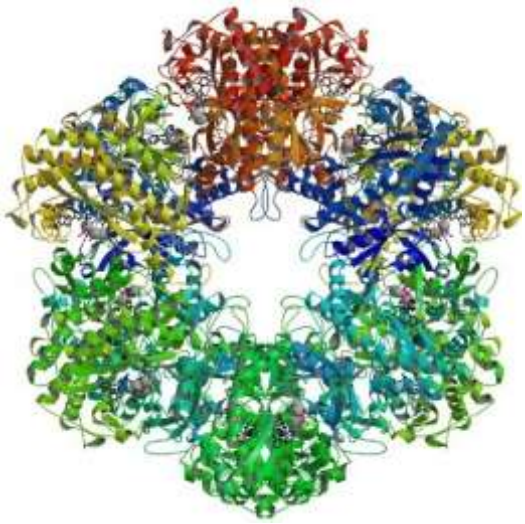


Figure 4 : Glutamine synthetase 1(3ZXR)[21]

Protein name	: Glutamine synthetase 1
Classification	: Ligase
Chains	: A, B, C, D, E, F
Total structure weight	: 332264.16
Length	: 486
Gene name	: glnA1 glnA Rv2220 MTCY190.31 MTCY427.01
Function	: catalyzes the ATP dependent condensation between glutamate and ammonia, to give glutamine.

M. tuberculosis in fact possesses four GS homologues, of which only one, the product of the *glnA1* gene is highly expressed and essential for the growth of the bacteria both *in vitro* and *in vivo*. In addition to its well-characterized role in **bacterial nitrogen metabolism**, *Mt*GS plays an important role in **cell wall biosynthesis**, specifically via the production of a poly-L-glutamate-glutamine component found exclusively in pathogenic mycobacteria.

Extracellular *Mt*GS may also affect pH modulation in phagosomes and consequently **prevent phagosome-lysosome fusion**. Numerous studies indicate that inhibition of *Mt*GS is a feasible therapeutic strategy. The extracellular location of the bulk of the enzyme furthermore obviates problems associated with the uptake of compounds across the notoriously impermeable mycobacterial cell wall [21].

MECHANISM :

GS catalyzes the ATP dependent condensation of glutamate with ammonia to yield glutamine. This mechanism takes place in two step:

The first step is the formation of the activated intermediate γ-glutamyl phosphate. The Mg^{2+} ion coordinates the γ-phosphate oxygen of ATP to allow phosphoryl transfer to the γ carboxylate group of glutamate, yielding the intermediate (acyl phosphate). ADP and Pi do not dissociate until ammonia binds and glutamine is released. The presence of ADP causes a conformational shift in GS that stabilizes the γ-glutamyl phosphate moiety.

This is followed by a second step deprotonation of ammonium, which allows ammonia to attack the intermediate from its nearby site to form glutamine[24].

The inhibition of GS secreted by M. tuberculosis is sufficient to halt the growth of the bacterium, suggesting that TB-GS might be a valid target for anti-tuberculosis drug-design. The structure of TB-GS is currently being solved to aid in the design of novel inhibitors for this enzyme [25].

The development of new classes of anti-tuberculosis drugs and new drug targets is of global importance, since attacking the bacterium using multiple strategies provides the best means to prevent resistance.

DRUG DISCOVERY[30]

Medicinal chemistry is the science that deals with the discovery and design of new therapeutic chemicals and their development into useful medicines. Medicinal chemistry involves synthesis of new molecules, investigation of the relationships between the structure of synthetic compounds and their biological activities, elucidations of their interactions with receptors of various kinds, including enzymes and DNA, the determination of their absorption, transport and distribution properties and studies of the metabolic transformations of these chemicals into other chemicals and their excretion.

The drug discovery process involves

- ✓ Designing
- ✓ Synthesizing
- ✓ Characterization
- ✓ Evaluation of new chemical entities
- ✓ Suitability for therapeutic use

It also includes study of existing drugs, their biological properties and their quantitative structural activity relationship (QSAR).

DRUG DESIGN[31]

Drug discovery process involves a rapid search for a small molecule often called as lead. Lead molecule is a chemical compound, which possess pharmacological or biological activity. Sources of lead compounds can come from natural sources, such as plants, animals, or fungi and also from synthetic chemical libraries.

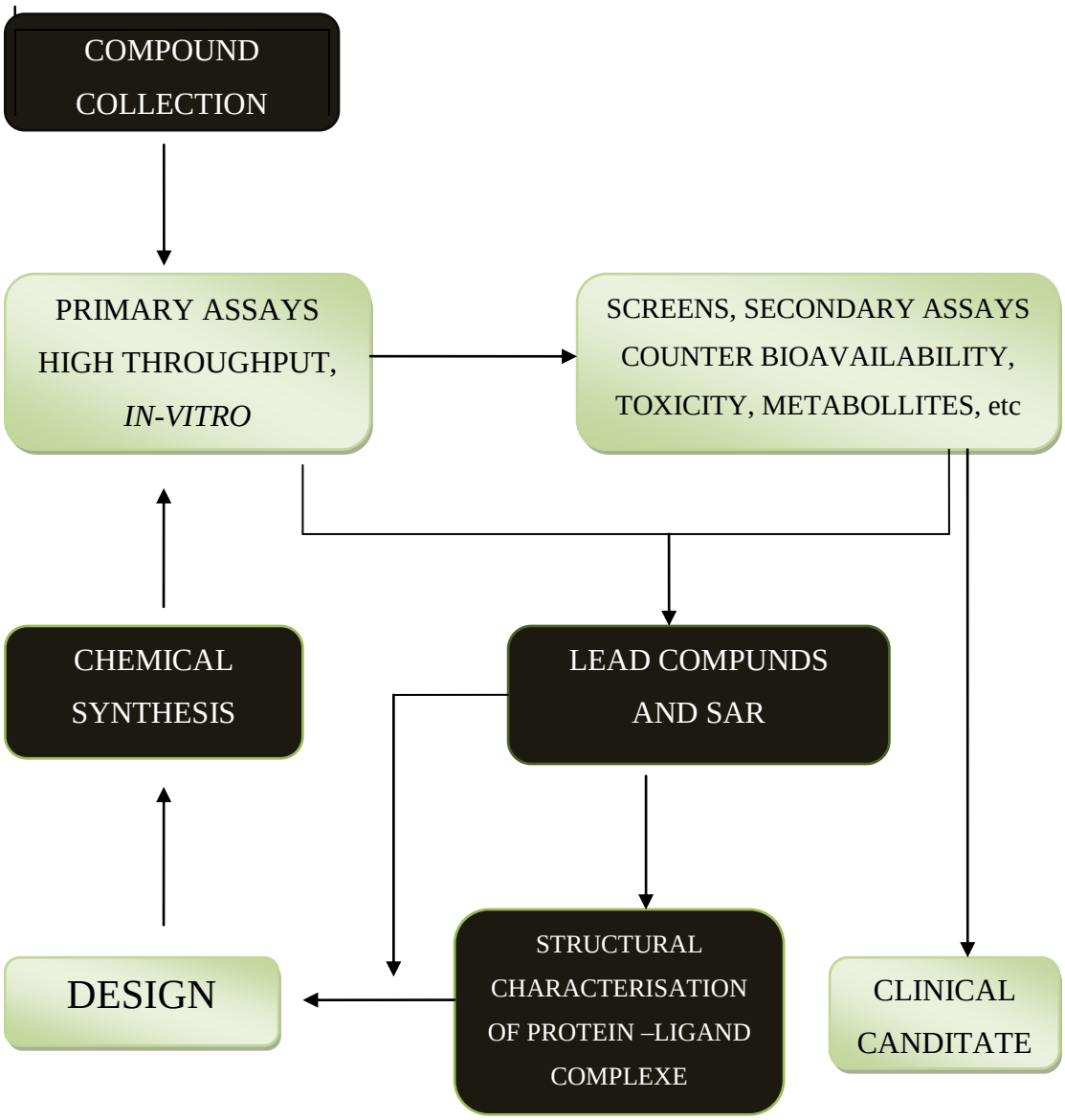


Figure :5 DRUG DISCOVERY CYCLE

LEAD OPTIMIZATION[30]

Newly pharmacologically active moieties may have poor drug-likeness and may require lead optimization step. This step involves chemical modification of a lead in order to improve their potency, selectively towards binding site, pharmacokinetic parameters and reduced toxicity.

COMPUTER AIDED DRUG DESIGN[31]:

The latest breakthroughs in computer-aided drug design, drug delivery systems, and enabling technologies. Computer Aided Drug Design (CADD) and Delivery Systems offers an in-depth discussion of the computer-assisted techniques used to discover, design, and optimize new, effective, and safe drugs.

Drug design, sometimes referred to as rational drug design or simply rational design, is the inventive process of finding new medications based on the knowledge of a biological target. The drug is most commonly an organic small molecule that activates or inhibits the function of a biomolecule such as a protein.

Drug design frequently but not necessarily relies on computer modeling techniques. This type of modeling is often referred to as computer-aided drug design. Finally, drug design that relies on the knowledge of the three-dimensional structure of the biomolecular target is known as structure-based drug design.

Furthermore, in vitro experiments complemented with computation methods are increasingly used in early drug discovery to select compounds with more favorable (absorption, distribution, metabolism, and excretion) and toxicological profiles.

BASIC NUCLEUS CHOOSEN FOR STUDY[33]:

Benzothiazole is a heterocyclic compound, weak base, having varied biological activities and still of great scientific interest now a days.

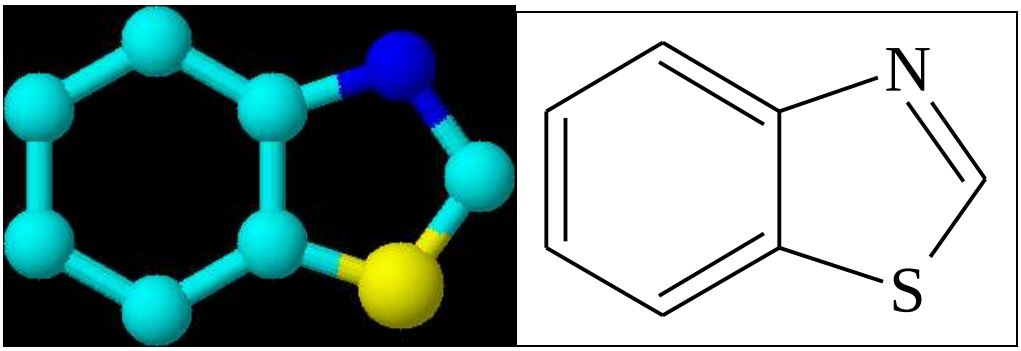


Figure 6 : **General structure of benzothiazole**

The Benzothiazole ring system bears phenyl ring fused with thiazole ring. Thiazole is a five membered heterocyclic ring system having sulfur and nitrogen as heteroatom. Benzothiazole is a privileged bicyclic ring system.

Benzothiazoles are an important class of privileged organic compounds of medicinal significance due to their recognized biological and therapeutic activities. Benzothiazole moites are part of compounds showing numerous biological activities such as

- ✓ Antimicrobial
- ✓ Anticancer
- ✓ Anthelmintic
- ✓ Anti-Diabetic
- ✓ Anticonvulsant
- ✓ Analgesic and Anti-Inflammatory Activities.

Due to its potent and significant biological activities, it has great pharmaceutical importance; hence, synthesis of derivatives is considerable interest. The small and simple benzothiazole nucleus if present in compounds involved in research aimed at evaluating new products that possess interesting biological activities [29].

REVIEW OF LITERATURE

On the basis of the design, benzothiazole has been identified as potential Anti-tubercular scaffold. It was therefore decided to conduct a literature survey of benzothiazole moieties and of the target glutamine synthetase

I. Reviews related to the target- Glutamine synthetase

1. **D.Eisenberg *et al.* (2000)**[22] carried out the glutamine synthetase as a regulated enzyme at the core of nitrogen metabolism, review the structural and functional studies of both bacterial and eukaryotic glutamine synthetase, with emphasis on enzymatic inhibitors.

2. **Marcus A. Horwitz *et al.* (2003)**[23] assessed the role of glutamine synthetase (GS), in the pathogenicity of mycobacterium tuberculosis; glnA1 was constructed via allelic exchange. The mutant had no detectable GS protein or GS activity and was auxotrophic for L-glutamine. In addition, the mutant was attenuated for intracellular growth in human THP-1 macrophages. Based on growth rates of the mutant in the presence of various concentrations of L-glutamine the importance of the enzyme was known. These studies demonstrate that glnA1 is essential for M.tuberculosis virulence.

3. **Wojciech W. Krajewski *et al.*(2008)**[24] summarized that glutamine synthetase catalyzes the ligation of glutamate and ammonia to form glutamine, with the hydrolysis of ATP. The enzyme is a central component of bacterial nitrogen metabolism and is a potential drug target. This study provides the first reported structure for a tauto form of the tuberculosis enzyme. The phospho compound, generated in situ by an active enzyme, mimics the phosphorylated tetrahedral adduct at the transition state.

4. **Berlicki L; Kafarski *et al.* (2008)**[25] studied the Glutamine synthetase enzyme which catalyses the formation of glutamine from glutamate and ammonium ion. It is one of the most important enzymes in nitrogen metabolism. The first part of the review presents the long-dating research on

inhibitors of glutamine synthetase. Analysis of their structure activity relationship is presented in some detail. The second part of the paper is dedicated to potential medical applications of glutamine synthetase inhibitors, which is proved to be effective anti-tuberculosis agent with high selectivity towards the pathogen.

5. **Mats Larhed *et al.* (2009)[26]** synthesized some potential anti-tubercular agents which targeted Glutamine Synthetase (GS), which is one of the latest targets of M.tb which catalyses the formation of glutamine from glutamic acid. In this work, novel GS inhibitors and new Palladium - catalyzed methods have been developed.

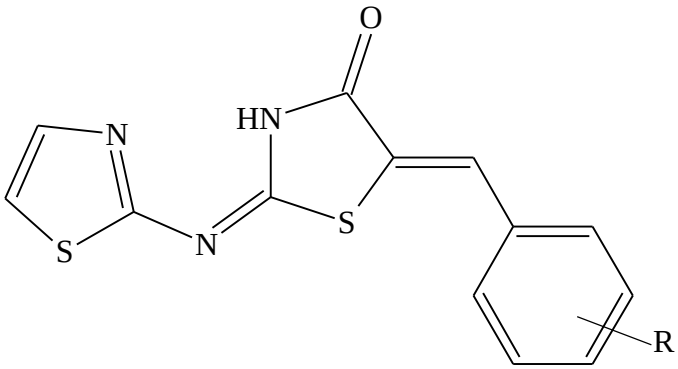
6. **Johan Gising *et al.* (2012)[27]** identified several classes of MtGS inhibitors targeting the ATP-binding site by a recent high-throughput screening study . They explored one of these classes, the 2-tert-butyl-4,5-diarylimidazoles, and presented the design, synthesis, and X-ray crystallographic studies leading to the identification of MtGS inhibitors at submicromolar IC50 values and promising antituberculosis MIC values.

7. **Luke R. Odell *et al.* (2014)[28]** presented an overview of the various strategies and compounds utilized to inhibit glutamine synthetase, a promising target for the development of anti TB drugs**.** The currently described inhibitors can be divided into two main classes, those that target the glutamate-binding site and ATP-competitive inhibitors. Compounds belonging to the first class are typically low molecular weight and polar analogues of glutamate, methionine sulfoximine or phosphinothricin.

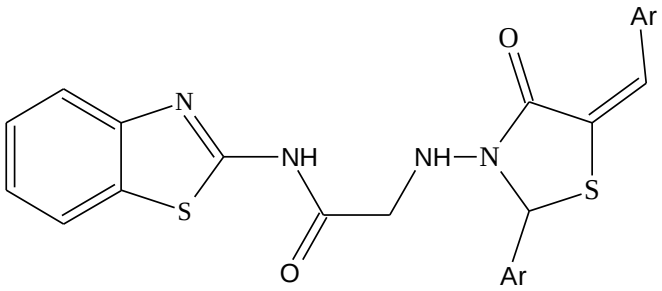
II. **Review related to benzothiazole nucleus and its biological activities**

8. **P Vicini *et al.* (2006)[36]** synthesized new 2-thiazolylimino-5-arylidene-4-thiazolidinones, unsubstituted or carrying hydroxy, methoxy, nitro and chloro groups on the benzene ring. They were assayed in vitro for their antimicrobial activity against Gram positive and Gram negative bacteria, yeasts and mould. The compounds were found to be very

potent towards all the tested Gram positive microorganisms (MIC ranging from 0.03 to 6 lμg/mL in most of the cases) and Gram negative Haemophilus influenzae (MIC 0.15–1.5 lμg/ mL), whereas they were ineffective against Gram negative Escherichia coli and fungi up to the concentration of 100μg/ml.

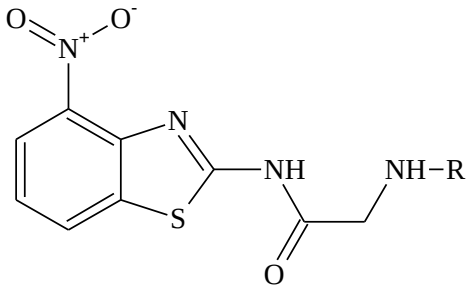


9. **J P Sen & S D Srivastava *et al.* (2008)** [37] carried out the systemic investigation of synthesis and biologically active compounds of 2 amino benzothiazole. Several new [(5-arylidine -2 aryl 4- oxo- 1,3-thiazolidine) 3-imino acetyl] 2 amino benzothiazole from 2 amino benzothiazole have been synthesized. All the synthesized products were evaluated for their antibacterial activity.

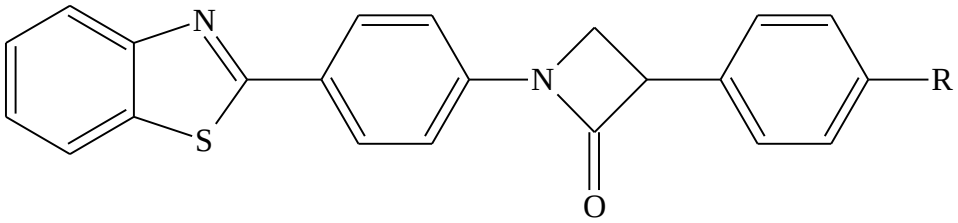


10. **Khalid A.Al Badraani *et al.* (2008)**[38] stated substituted anilines are readily converted to 2-amino substituted benzothiazole by reaction with Potassium thiocynate and bromine in glacial acetic acid. The product 4-nitro-2-amino benzothiazole Acetyl chloride was allowed to react with various aryl amine to give aryl aminoacetyl -2-amino-4-nitrobenzothiazole derivatives. The reaction of hydrazine hydrate and ethyl -3-amino beridyl acetate gave the acid hydrizide. The synthesized compounds were

characterized on the basis of IR spectral analysis and the results found compatible with their assigned structures.

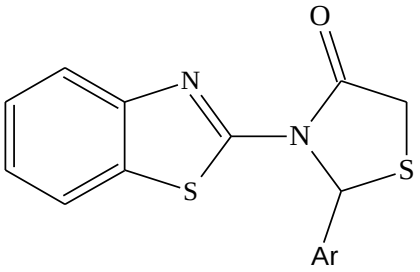


11. **SR Reddy *et al.* (2010)**[39] described the synthesis of benzothiazole appended β-lactams. This methodology involves [2+2]-cycloaddition of benzothiazole substituted imines with chloroacetyl chloride in the presence of triethylamine to yield the corresponding product (substituted azetidine 2 one derivatives)in moderate to good yields. The reaction is compatible with a wide varity of functional groups.

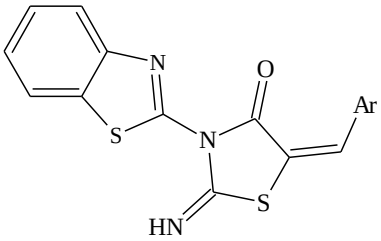


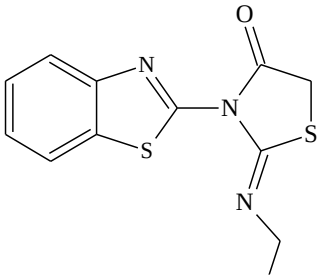
12. **Taleb H. Al-Tel *et al.* (2011)**[40] studied new antimicrobial agents, imidazo[1,2-a]pyridine and imidazo[2,1-b][1,3]benzothiazole Results from this study showed that the nature of the substituents on the armed aryl groups determines the extent of the activity of the fused imidazopyridine and/or imidazobenzothiazole derivatives. Preliminary structure activity observations revealed that bromofluoro substituents enhanced the antimicrobial activity significantly compared to other substituents.

13. **Pareek *et al.* (2014)**[43] synthesized the substituted-2-aryl-3-(benzothiazolyl)-1,3-thiazolidine-4-one. The synthesized compounds were screened for their anti-inflammatory, antiulcer, antitumor, entomological and antibacterial activities.



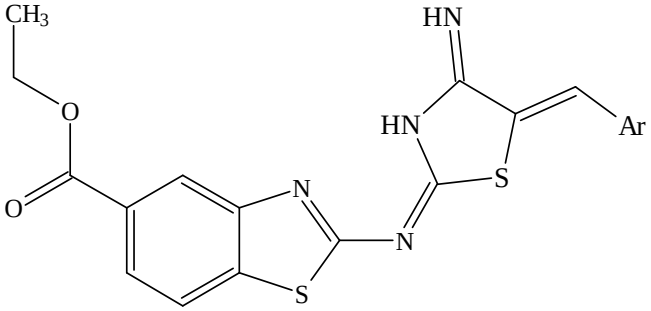
14. **Loah R. Hemeda *et al.* (2015)[44]** have reviewed the synthesis and different biological activities of some derivatives of 5-arylidene derivatives of benzothiazol-2-yl substituted 2- imino thiazolidin-4-ones and N-Mannich bases of N-substituted-2-Iminothiazolidin-4-ones.





NR_1R

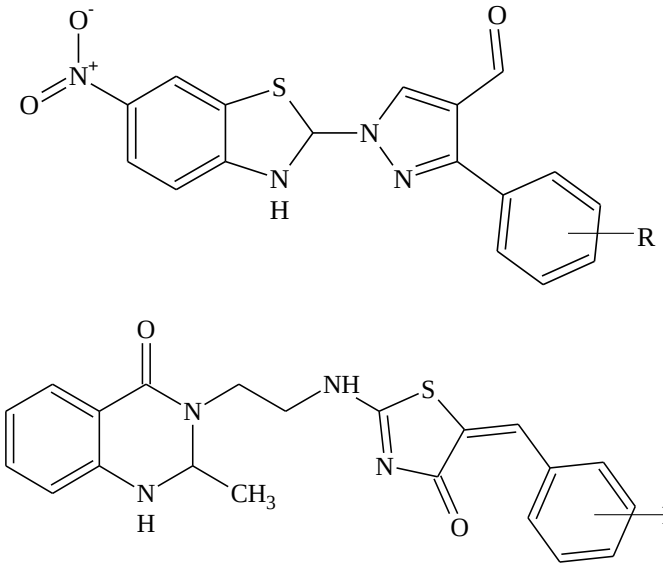
15. **Khaled R.A.Abdellatif *et al.* (2015)[45]** synthesised new 5-arylidene thiazolidinone derivatives and evaluated for their potential as antitumor lead compounds. These compounds were tested in vitro on human breast MCF-7 and non-small cell lung A549 cancer cell lines which showed potent antitumor activity in the micro molar level. All the prepared compounds were docked against EGFR using 4-anilinoquinazoline inhibitor (PDB ID: IM17) in away to explore their binding mode.



Ar = (a)-2-thionyl ;(b)- 3-$(OCH_3)C_6H_5$

III. Review of the several works on benzothiazole, few of them are enlisted here in support of their antitubercular activity

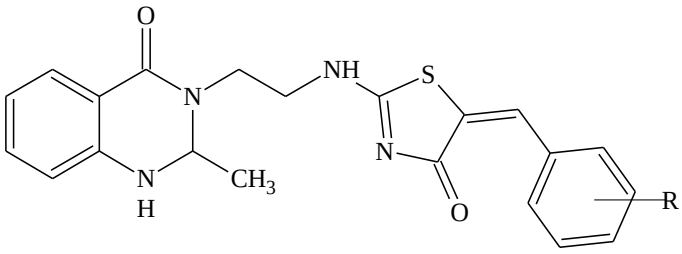
16. **A.A.Chowki *et al.* (2008)[46]** synthesized various 6-nitro-2-[4-formyl-3-(substituted phenyl) pyrazol-1-yl] benzothiazole and screened them for antitubercular activity against H37Rv strain of Mycobacterium tuberculosis. Synthesis of 6-Nitro-2-[4-formyl-3-(substituted phenyl) pyrazol-1-yl] benzothiazoles reported in this study provides a novel example of Vilsmeier Haack reagent mediated heterocyclic synthesis. 6-Nitro-2-[4-formyl-3-(substituted phenyl) pyrazol-1-yl] Benzothiazoles exhibit a range of activities in the antitubercular screens. Many of these derivatives exhibited activity comparable to that of the standards.



17. **B.S. Sathe *et al.* (2010)[47]** treated 4-Fluoro-3-chloroanilline with Potassium thiocyanate in presence of Glacial acetic acid and bromine to synthesise 2-amino-6-fluoro-7-chlorobenzothiazole. The synthesized compound in presence of m-nitro benzaldehyde refluxed in ethanol gave 3-[6'-fluoro-7'-chloro(1',3') benzothiazol-2'-yl]-m-nitrophenyl(1,3) thiazolidine-4-one. This compound was treated with ortho, meta and para nitroanillines, ortho, meta, para chloroanillines, morpholino, Piperazine and diphenylamine in the presence of DMF to obtain different derivatives. Some of these derivatives showed promising antimycobacterial activity.

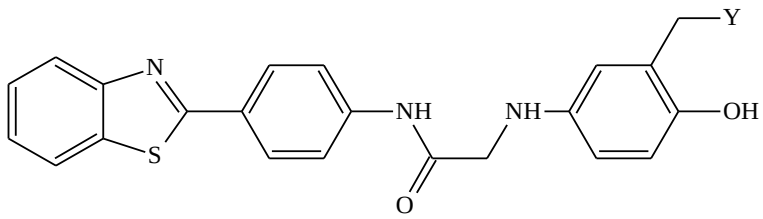
18. **Chakraborti *et al.*(2014)[48]** reported that benzothiazole-2-carboxyaryl alkyl ssamides as new class of potent anti-mycobacterial agents. Forty one target compounds have been synthesized following a green synthetic strategy using water as the reaction medium to construct the benzothiazole scaffold followed by microwave-assisted catalyst-free, and ammonium chloride-catalysed solvent-free amide coupling. The antimycobacterial potency of these compounds were determined against H37Rv strain. Twelve compounds exhibited promising anti-TB activity in the range of 0.78-6.25 μg/mL and were found to be non-toxic(<50% inhibition at 50μg/ml) to HEK 20 293T cell lines with therapeutic index of 8-64.

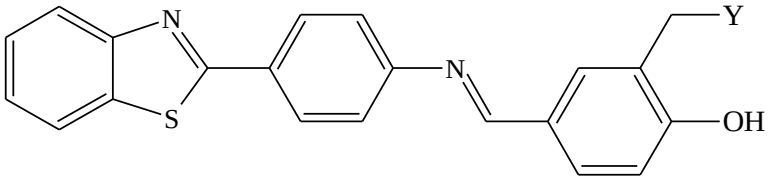
19. **H Chikhalia *et al.* (2014)[49]** synthesized and studied a series of quinazolinone based 5-arylidine-2-(2-methyl-4-oxoquinazlin-3-(4H)-yl) ethyl) amino) thiazol-4 (5H)-one derivatives. Quinazoline and styryl thiazolidinones have been clubbed through ethyl linkage to get hybrid molecules. The final synthesized compounds were screened for their in vitro antimicrobial activity against bacterial fungal strains. In vitro antimycobacterial efficacy has also been studied against *Mycobacterium tuberculosis* H37Rv using BACTEC MGIT method.



20. **Vinayak *et al.* (2015)[50]** reacted 2-Amino-4/6-substitutedbenzothiazoles on reaction with substituted aromatic aldehydes which gave 2-(arylidenimino)-4/6-substituted benzothiazoles , which on reaction with mercaptoacetic acid provide 2-aryl-3-(substituted benzothiazolyl)-1,3-thiazolidine-4-ones. All the newly synthesized compounds were screened for antibacterial activity at a concentration of 200 μg/mL and 100 μg/mL using DMF as a control and Streptomycin and Ceftazidime used as standard against gram positive and

gram negative bacteria**.**



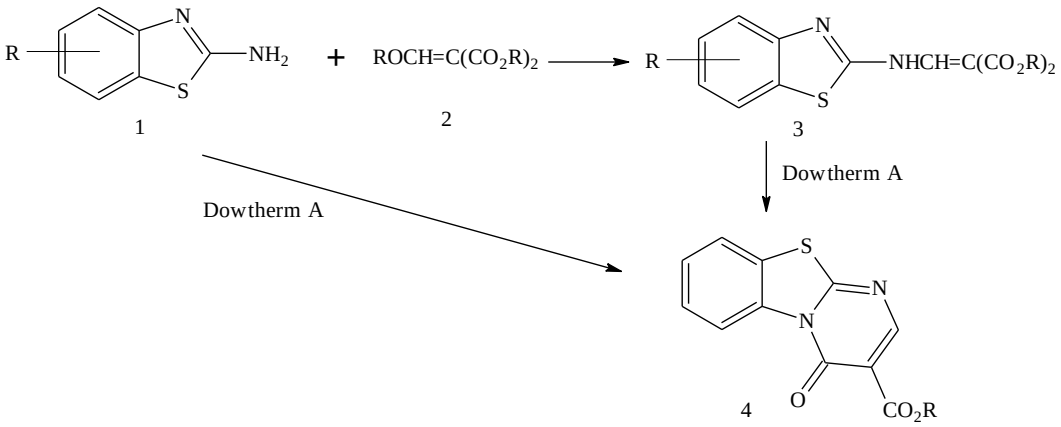


Y = $N(C_2H_5)_2$; 4-methyl piperidin-1-yl

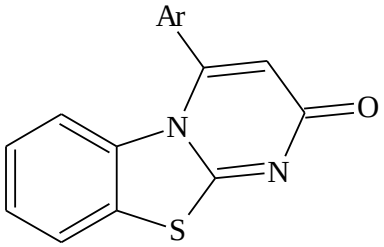
IV. Reviews related to the synthetic procedure:

Scheme 1:

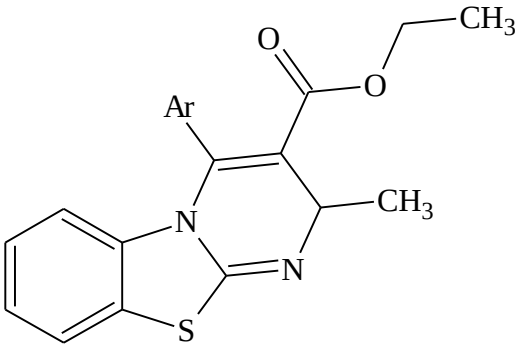
21. **Robert J. Alaimo (1973)[51]** explained the synthesis of the 4H-pyrimmido[2,1-b]benzothiazole-4-ones(4). It involves the reaction of suitability substituted 2aminobenzothiazoles(1) with alkoxymethylenemalonatee esters(2). When the initial condensation was carried out in alcohol, the product was the corresponding 2-(benzothiazolyl) aminomethylene malonate(3),(5). These intermediates were readily cyclized to the corresponding pyrimido[2,1-b]benzothiaoles in hot Dowtherm A. However since the complete reaction could be accomplished more conveniently in one step by heating a mixture of (1) and (2) in Dowtherm A until the theoretical amount of alcohol has been collected, the compounds(4) were prepared by this method.



22. **Saeed Balalaie *et al.* (2012)[52]** carried out one-pot three-component reaction of 2-aminobenzothiazole, benzaldehyde derivatives and *β*-ketoester, *β*-diketone or malonate derivatives in solvent-free conditions, to yielded pyrimido[2,1-*b*]benzothiazole derivatives at 60°C in 60% - 72% yields without using any catalyst.

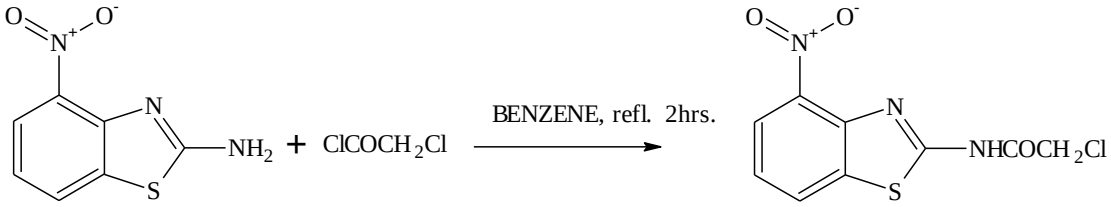


23. **P K Sahu *et al.* (2014)[53]** developed an efficient method for the preparation of 4*H*-pyrimido [2,1-b]benzothiazole derivatives by the condensation of aldehydes, β-keto ester, and 2-amino benzothiazole under solvent and solvent free conditions using various catalysts. Good yield was obtained at 60-65^{0}C under solvent-free conditions. This study suggests that acetic acid and metal catalysts follow different mechanism.

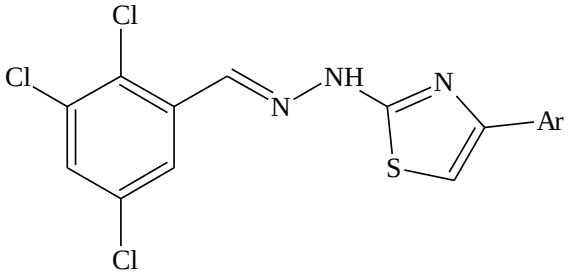


Scheme 2:

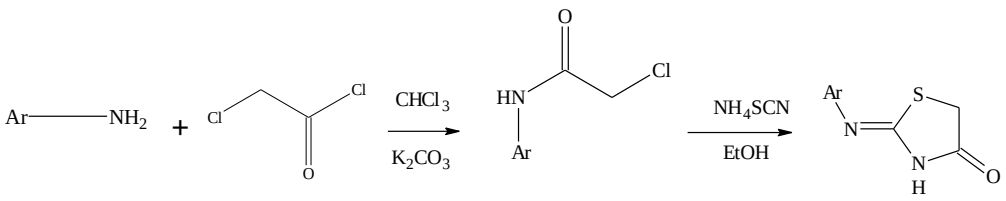
24. **Khalid A.Al Badraani *et al.* (2008)[38]** connected substituted anilines to 2-amino substituted benzothiazole by reaction with Potassium thiocynate and bromine in glacial acetic acid. The -4-nitro-2-amino benzothiazole acetyl chloride obtained from reaction between -4-nitro-2-amino benzothiazole with chloro acetyl chloride.



25. **BS Holla *et al.* (2008)[54]** synthesized the series of novel 4-aryl/chloroalkyl-2-(2,3,5-trichlorophenyl)-1,3-thiazoles by condensing 2,3,5-trichlorobenzenecarbothioamide with phenacyl bromide/ dichloroacetone. 2,3,5-Trichlorobenzaldehyde thiosemicarbazone on treatment with phenacyl bromide afforded 4-aryl-2-(2,3,5-trichlorophenylidenehydrazino)-1,3-thiazoles (10aeg) in good yield. These compounds were also screened for their antibacterial and antifungal activities.

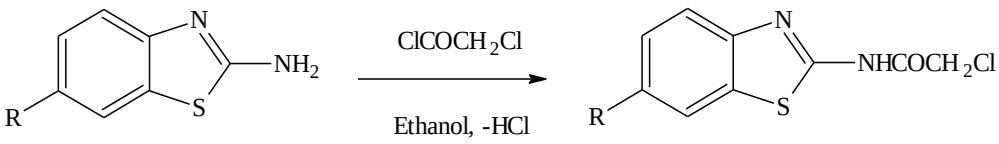


26. **Haider Behbehani *et al.* (2011)[55]** The 4-thiazolidinones were used as key intermediates for the synthesis of 2-arylimino-5-arylidene-4-thiazolidinones derivatives *via* nucleophilic addition reactions with the arylidene malononitrile. The 4-thiazolidinone reacted with the benzenediazonium chloride to afford the arylhydrazones.



27. **Navin B Patel and Sarvil D. Patel *et al.* (2012)**[56] synthesized 2-Chloromethyl-5- substituted phenyl-1,3,4-oxadiazoles then coupled with phenolic group of 4-hydroxy aniline, further converted to 2-chloro-*N*-substituted phenyl acetamides on reaction with chloro acetyl chloride and finally cyclized with ammonium thiocynate to form targeted compounds.

28. **Dhamak Kiran Bhausaheb *et al.* (2015)**[57] synthesized the derivatives of benzothiazoles and evaluated for their antifungal activity. 2-amino benzothiazole was first converted to 6 substituted derivatives of 2-amino benzothiazole by nitration and bromination reaction to yield 6-nitro-2-amino benzothiazole and 6-bromo-2-aminobenzothiazole respectively. All the derivatives including 2-amino benzothiazole were further treated with chloroacetyl chloride to form chloroacetamido derivatives of benzothiazole. Further, the product was treated with various heterocyclic and aromatic amines. Synthesized substituted benzothiazole derivatives were investigated for their antifungal activity.



6-methyl-1,3-benzothiazol-2-amine

2-chloro-*N*-(6-methyl-1,3-benzothiazol-2-yl)acetamide

V. Reviews related to biological evaluation of Antitubercular activity by MABA

29. **Collins and Franzblau *et al.* (1997)** In response to the need for rapid, inexpensive, high-throughput assays for antimycobacterial drug screening, a

microplate-based assay which uses Alamar blue reagent for determination of growth was evaluated. They concluded MABA is sensitive, rapid and nonradiometric and offers the potential for screening, with or without analytical instrumentation, large numbers of antimicrobial compounds against slow-growing mycobacteria.

30. **Robin K Pettit *et al.* (2005)** explained about the redox indicator Alamar blue has been used extensively in planktonic bacterial and fungal susceptibility assays and mammalian cell culture cytotoxicity assays. Alamar blue is reduced by FMNH2, FADH2, NADH, NADPH, and cytochromes. Alamar blue both fluoresces and changes color in response to chemical reduction, and the extent of the conversion is a reflection of cell viability. Alamar blue is water soluble, so cell proliferation assays are not required. Data may be collected with either fluorescence-based or absorbance-based instruments.

31. **Jose d Jesus Alba-Romero *et al.* (2011)** applied the Alamar blue assay to determine the susceptibility to anti-tuberculosis pharmaceuticals. The results showed that the MABA test is fast and easy to apply. It is very reliable method to determining the drug susceptibility to pharmaceuticals.

32. **Sephra N.Ramprasad *et al.* (2012)** studied the various applications of Alamar blue as an indicator. Alamar blue is an indicator that is used to evaluates metabolic function and cellular health. The Alamar blue bioassay is being utilized to access cell viability and cytotoxicity in a biological and environmental system and in a number of cell types including bacteria, yeast, fungi, and protozoa.

33. **Franck Bonnier *et al.* (2015**) indicated the comparisons of 2D and 3D cell culture models differences in cellular morphology and metabolism, commonly attributed the better representation of *invivo* conditions of the latter cell culture environment. Using the well established Alamar Blue assay, the study demonstrates how the transfer from 2D substrates to 3D

collagen matrices can affect the uptake of the resazurin itself, affecting the outcome of the assay. Using flow cytometry, it is demonstrated that the cell viability is unaffected when cells are grown on collagen matrices.

AIM AND OBJECTIVES

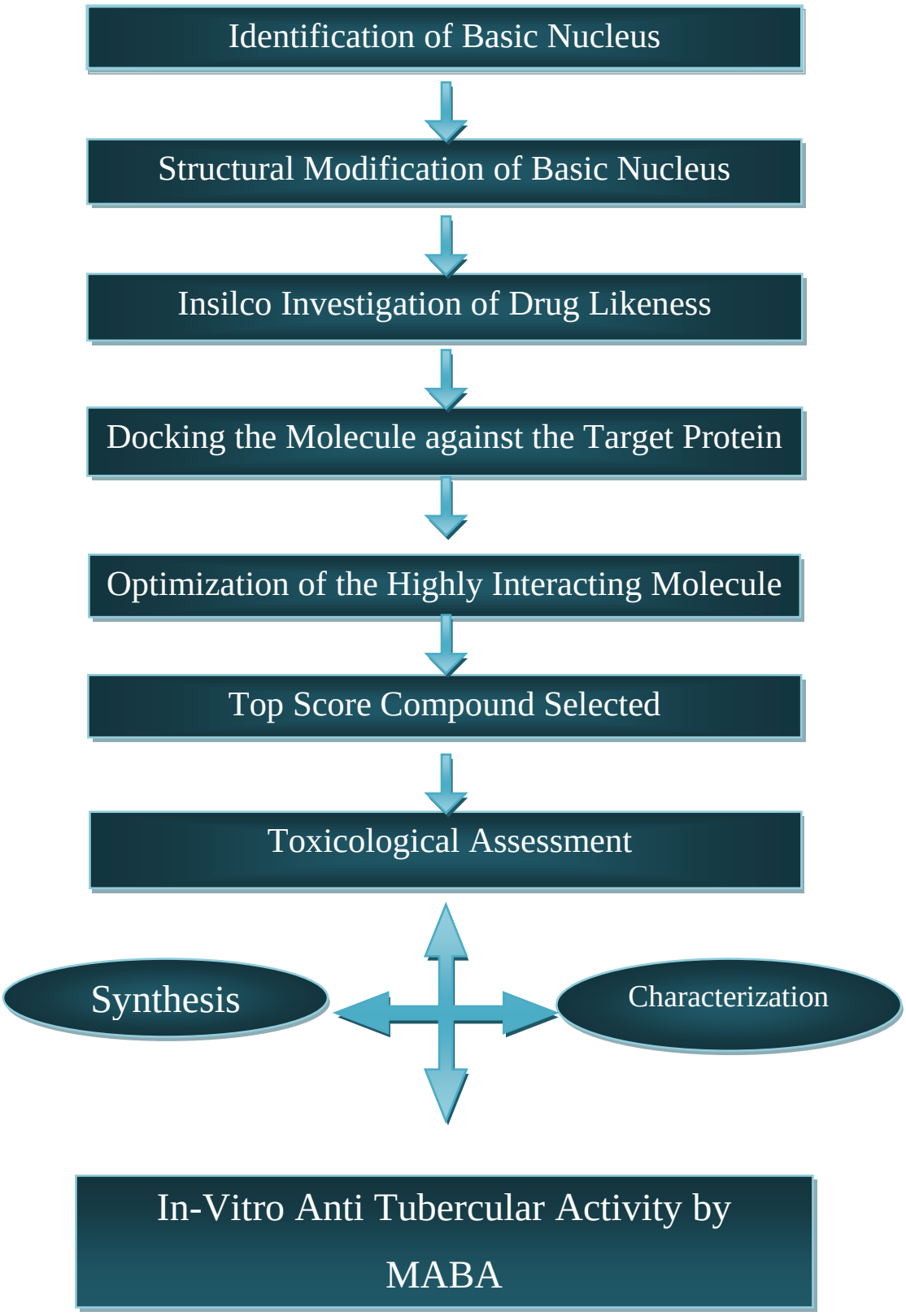
AIM:

The aim of this project is to design molecules into potential anti-tubercular activity that is capable of inhibiting cell wall synthesis by inhibiting glutamine synthetase. The designed compounds will be synthesized, characterized and evaluated for activity and toxicity.

OBJECTIVE:

- To design the molecule and docked against a specific crucial target, Glutamine Synthetase I, which is involved in the cell wall biosynthesis and nitrogen metabolism.
- In-silico prediction of Drug Likeness and Toxicity
- Laboratory synthesis of the top G score compounds
- Melting point determination and Thin layer Chromatography
- Characterization of the synthesized compounds by
    - Infrared Spectroscopy
    - Nuclear Magnetic Resonance Spectroscopy
    - Mass Spectroscopy
- Determination of *in vitro* anti tubercular activity of synthesized compounds

The present study will be conducted according to the following design



PLAN OF WORK

- The main aim of this project is to synthesize a novel compounds containing anti tubercular activity.
- The toxicity of compounds are visualized by OSIRIS®.
- The selected enzyme is docking against compounds which is to be synthesized.
- The selected compounds were synthesized at appropriate manner then purified by recrystallization, characterized by sharp melting point and Thin Layer Chromatography.
- The following compounds are confirmed by characterized techniques which is hyphenated like, GC-MS and LC-MS analysis(if necessary).In this technique, molecular weight and purity of the formed compounds are determined.
- The molecular structure of the compounds is interpreted by proton NMR (1HNMR) and the functional groups of the compounds are analyzed by IR studies.
- The anti-tubercular activity of the compounds are evaluated by MABA

Finally, a novel and potent anti-tubercular activity containing compounds are synthesized, purified, characterized and evaluated.

MATERIALS AND METHODS

4.1 COMPUTER AIDED DRUG DESIGN [66]

A binding interaction between a small molecule ligand and an enzyme protein results in activation or inhibition of the enzyme, which results in agonism or antagonism.

Identification of new ligands for a given receptor by searching large databases of 3D structures of small molecules to find those fitting the binding pocket of the receptor using docking programs. This method is known as virtual screening.

DOCKING

Docking program is used to fit the ligand molecule into the target structure in a variety of position, conformations and orientations. Docking mode is known as pose. Each pose scored based on its complementarities to the target in terms of shape and properties such as electrostatics in order to identify the most favorable energetic pose.

SCORING FUNCTIONS[67]

One early general-purposed empirical scoring function to describe the binding energy of ligands to receptors was developed by Böhm. This empirical scoring function took the form:

- ΔG_0 – empirically derived that in part corresponds to the overall loss of translational and rotational entropy of the ligand upon binding.
- ΔG_{hb} – contribution from hydrogen bonding
- ΔG_{ionic} – contribution from ionic interactions

- ΔG_{lip} – contribution from lipophilic interactions where is surface area of lipophilic contact between the ligand and receptor
- ΔG_{rot} – entropy penalty due to freezing a rotatable bond in the ligand bond upon binding

$$\Delta G_{bind} = -RT \ln K_d$$

$$K_d = \frac{[Ligand]\,[Receptor]}{[Complex]}$$

$$\Delta G_{bind} = \Delta G_{desolvation} + \Delta G_{motion} + \Delta G_{configuration} + \Delta G_{interaction}$$

Where:

- $\Delta G_{desolvation}$ is the enthalpic penalty for removing the ligand from solvent
- ΔG_{motion} is the entropic penalty for reducing the degrees of freedom when a ligand binds to its receptor
- $\Delta G_{configuration}$ is the conformational strain energy required to put the ligand in its "active" conformation
- $\Delta G_{interaction}$ is the enthalpic gain for "resolvating" the ligand with its receptor

According to Gibbs free energy equation, the relation between dissociation equilibrium constant, Kd, and the components of free energy was built.

Molecular Docking by AUTODOCK® [74,75]

AutoDock® 4.2.5.1 is an automated procedure for predicting the interaction of ligands with biomacromolecular targets. Progress in biomolecular x-ray crystallography continues to provide important protein and nucleic acid structures. These structures could be targets for bioactive agents in the control of animal and plant diseases, or simply key to the understanding of fundamental aspects of biology. The precise interaction of such agents or candidate molecules with their targets is important in the drug discovery process.

In any docking scheme, two conflicting requirements must be balanced: the desire for a robust and accurate procedure, and the desire to keep the computational demands at a reasonable level. The ideal procedure would find the global minimum in the interaction energy between the substrate and the target protein and exploring all available degrees of freedom (DOF) for the system. AutoDock® combines two methods to achieve these goals: rapid grid-based energy evaluation and efficient search of torsional freedom.

The current version of AutoDock® using the Lamarckian Genetic Algorithm and empirical free energy scoring function, typically will provide reproducible docking results for ligands with approximately 10 flexible bonds.

The quality of any docking results depends on the starting structure of both the protein and the potential ligand. The protein and ligand structure need to be prepared to achieve the best docking results.

- **Protein preparation**
- **Ligand preparation**
- **Receptor grid generation**
- **Ligand docking (screening)**

DOCKING PROCEDURE

Preparation of protein:

- Read molecule from the file (allows reading of PDB coordinate files.)
- Edit -Charges – Compute Gasteiger (for arbitrary molecules)
- Edit – Hydrogen –Merge non polar
- Save as **.pdb** in AutoDock® folder

Preparation of Ligand:

- Ligand –Input from file
- Ligand – Torsion –choose torsion: Rotatable bonds are shown in green, and non-rotatable bonds are shown in red. Bonds that are potentially rotatable but treated as rigid, such as amide bonds and bonds that are made rigid by the user, are shown in magenta.
- Ligand – Torsion –set number of torsion: sets the number of rotatable bonds in the ligand by leaving the specified number of bonds as rotatable.
- Ligand – Output – save as **.pdbqt** in AutoDock folder

Grid preparation:

- Grid – Macromolecule -open (open the pdb file thet has been saved and then save it in **pdbqt** extension in AutoDock folder)
- Grid – Set map types –open ligand : tools to define the atom types for the grids that will be calculated
- Grid – Grid box – launches interactive commands for setting the grid dimensions and center (Set dimension of 60 **x** 60 **x** 60 – Center: center on macromolecule)
- File – Close saving current
- Grid – Output – save as **.gpf** (grid parameter file)
- Open command prompt **[cd AutoDock**

  cd 4.2.5.1

  autogrid4.exe –p a.gpf –l a.glg]

Preparation of Docking Parameters:

- Docking –Open the macromolecules – set rigid file name.
- Docking – ligand – open the ligand.
- Docking –search parameters – genetic algorithm parameters : this command opens a panel for setting the parameters used by each of the search algorithms, such as temperature schedules in simulated annealing and mutation/crossover rates in genetic algorithms.
- Docking – docking parameters: opens a panel for setting the parameters used during the docking calculation, including options for the random number generator, options for the force field, step sizes taken when generating new conformations, and output options.
- Docking- output –Lamarkian GA –save as **.dpf (**docking parameter file**)**
- Open command prompt **[autodock4.exe –p a.dpf –l a.dlg]**

Visualization / Interpretation of Docking

- Analysis –Docking – open .dlg (docking log file) file
- Analysis – open the macromolecule
- Analysis – Confirmation –Play and Play ranked by energy : Play- will use the order of conformations as they were found in the docking calculations, and Play Ranked By Energy will order the conformations from lowest energy to highest energy.
- Analysis – Load : Information on the predicted interaction energy is shown at the top and the individual conformations
- Analysis – Docking – show interaction: specialized visualization to highlight interactions between the docked conformation of the ligand and the receptor.

LIPINSKI'S RULE[68]

Lipinski's rule of five is a rule of thumb to evaluate drug likeness, ie., to determine if a chemical compound with a certain pharmacological or biological activity has the properties that would make it a likely orally active drug in humans. Christopher A. Lipinski formulated the rule in 1997, based on the observation that most medication drugs are relatively small and lipophilic molecules.

The rule describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion (ADME). However, the rule does not predict if a compound is pharmacologically active.

The rule is important for the drug development where a pharmacologically active lead structure is optimized step-wise for increased activity and selectivity, as well as drug-like properties as described by Lipinski's rule. The modification of the molecular structure often leads to drugs with higher molecular weight, more rings, more rotatable bonds, and a higher lipophilicity. Lipinski's rule says that, an orally active drug has no more than one violation of the following criteria:

- Partition coefficient of log P less than 5
- Not more than 5 hydrogen bond donors (nitrogen or oxygen atoms with one or more hydrogen atoms)
- Not more than 10 hydrogen bond acceptors (nitrogen or oxygen atoms)
- Molecular weight under 500 daltons
- An additional rule was proposed by **Veber**

  < 10 rotatable bonds

IN-SILICO TOXICITY PREDICTION[73]

OSIRIS® :

In silico toxicity prediction is done using **OSIRIS®** Property Explorer. It is a free software available for access in the Organic Chemistry Portal. Using this prediction tool, mutagenicity, tumerogenicity, skin irritation and reproductive effects can be calculated.

The prediction properties relies on a precompiled set of structure fragment that gives rises to toxicity alerts in case they are encountered in the structure currently drawn. These fragment lists is created by rigorously shredding all compounds in the data base known to be active in a certain toxicity class. During the shredding any molecule is first cut at every rotatable bonds leading to a set of core fragments.

MOLINSPIRATION® :

The designed and docked molecules are screened in silico using **MOLINSPIRATION®** Cheminformatics software to evaluate drug likeness. This tool is quick and easy to use. It is a software available online for calculation of important molecular properties log P, polar surface area, number of hydrogen bond donors and acceptors and others, as well as prediction of bioavailability score for the most important drug targets (GPCR ligands, Kinase inhibitors, ion channel modulators, nuclear receptors).

4.2 SYNTHETIC METHODOLOGY

Scheme 1:

General Procedure for the Synthesis of 4*H*-Pyrimido [2, 1-*b*] benzothiazole derivatives:

A mixture of 2-aminobenzothiazole (0.01mol) and aromatic aldehyde derivatives (0.01mol) and β keto ester (0.01mol) were heated at 60°C in the solvent-free conditions for 5 - 6 hr. Completion of the reaction was confirmed by TLC (Petroleum ether: EtOAc 1:4). At the end of the reaction, the mixture was washed 3 times (3 × 20 ml) with water and diethyl ether.

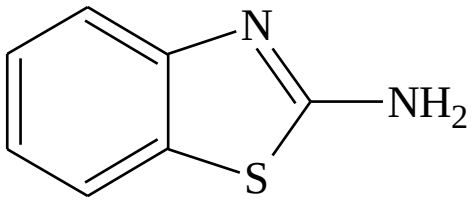
Scheme 2:

General Procedure for the Synthesis of Imino-3-(6-substitutedbenzo[d]thiazol-2-yl) thiazolidin-4-one:

2 amino benzothiazole (0.01mol) and chloroacetyl chloride (0.01mol) in dry benzene (50ml) in the presence of potassium carbonate (0.15mol) was refluxed for 12 hours. The mixture was stirred with water (100 mL) and filtered. The solid product was then washed with 5% $NaHCO_3$ solution and subsequently with water. The crude product was dried and crystallized from ethanol. Then the product of the first (0.01mol) was added to ammonium thiocynate (0.03mol) in ethanol (30ml) and heated under reflux for 5 hours at 120^0C and then kept overnight. Then the sample was collected then washed with water and recrystallized the product with ethanol.

REACTANT PROFILE

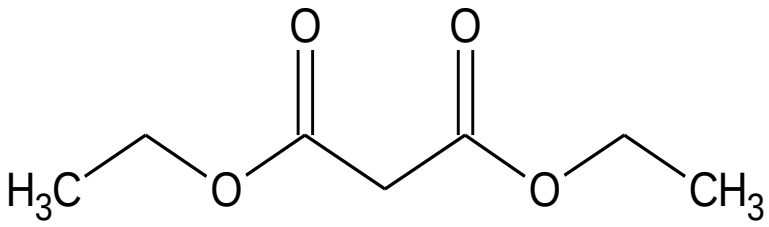
2 Amino Benzothiazole:



1, 3-benzothiazol-2-amine

SYNONYM	1, 3-benzothiazol-2-amine
MOLECULAR FORMULA	$C_7H_6N_2S$
MOLECULAR WEIGHT	150.20
MELTING POINT	$126\text{-}129^0C$
DESCRIPTION	Pale white colour powder

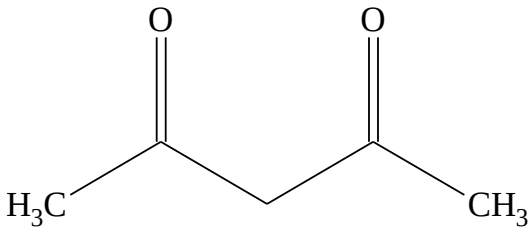
Diethyl Malonate:



Diethyl malonate

SYNONYM	1,3-Diethyl propanedioate
MOLECULAR FORMULA	$C_7H_{12}O_4$
MOLECULAR WEIGHT	160.16
BOILING POINT	199^0C
DESCRIPTION	Colourless liquid

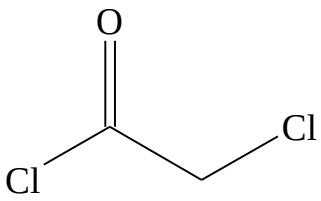
Acetyl acetone:



pentane-2,4-dione

SYNONYM	Pentane-2, 4-dione
MOLECULAR FORMULA	$C_5H_8O_2$
MOLECULAR WEIGHT	100.12
BOILING POINT	140^0C
DESCRIPTION	Reddish brown colour liquid

Chloro Acetyl Chloride:



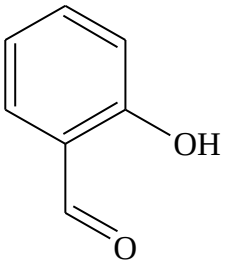
chloroacetyl chloride

SYNONYM	Chloroacetyl chloride
MOLECULAR FORMULA	$C_2H_2Cl_2O$
MOLECULAR WEIGHT	112.192
BOILING POINT	140^0C
DESCRIPTION	Colourless liquid

Ammonium thiocynate:

MOLECULAR FORMULA	NH_4SCN
MOLECULAR WEIGHT	76.122
BOILING POINT	170^0C
DESCRIPTION	Colourless hygroscopic crystalline solid

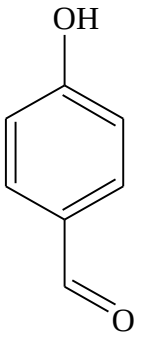
Salicylaldehyde:



salicylaldehyde

MOLECULAR FORMULA	$C_7H_6O_2$
MOLECULAR WEIGHT	122.121
BOILING POINT	$196\text{-}197^0C$
DESCRIPTION	Colourless liquid

4 –OH Benzaldehyde:



4-hydroxybenzaldehyde

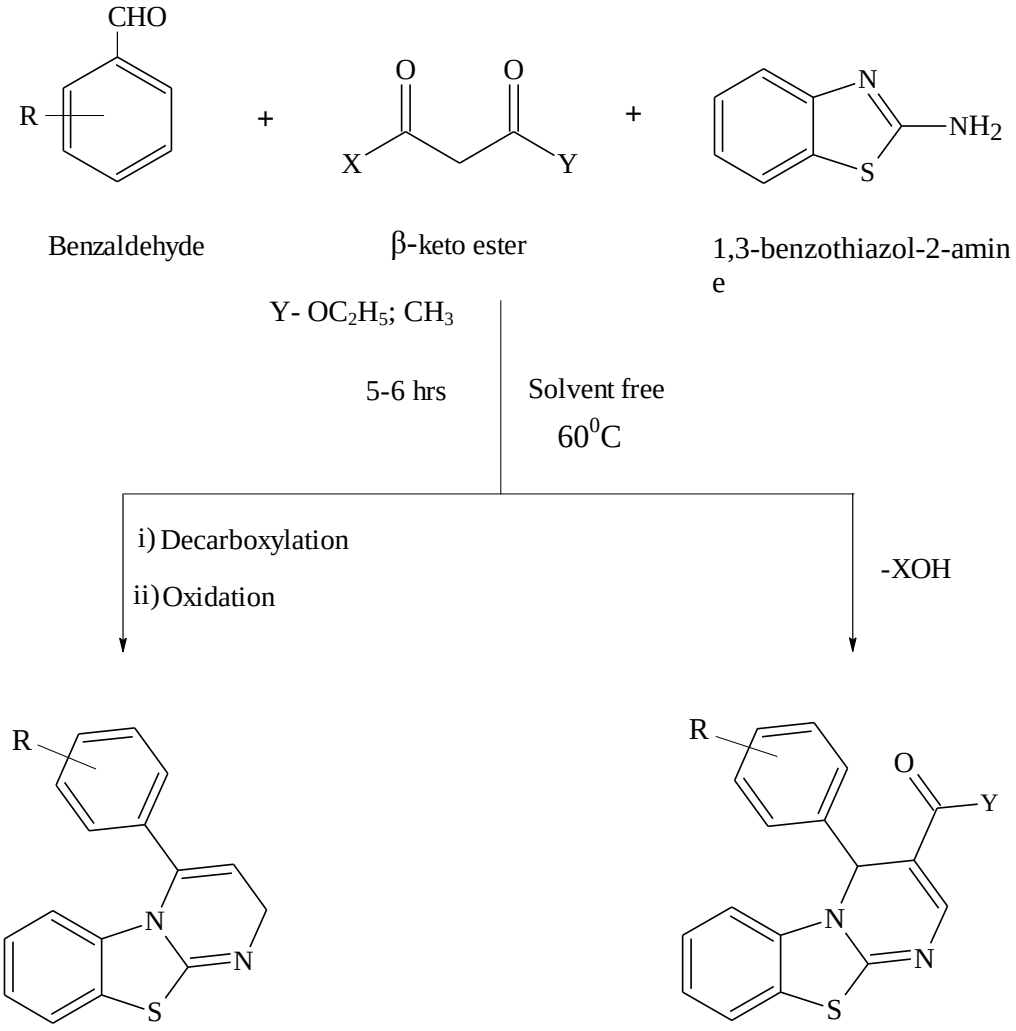
MOLECULAR FORMULA	$C_7H_6O_2$
MOLECULAR WEIGHT	122.121
BOILING POINT	$112\text{-}116^0C$
DESCRIPTION	White powder

SYNTHETIC DERIVATIVES:

A. 4*H*-pyrimido[2,1-*b*][1,3]benzothiazole derivatives

B. 5-Arylidene-2-imino-3-(benzo[d]thiazol-2-yl) thiazolidine-4- one

Reaction: 1

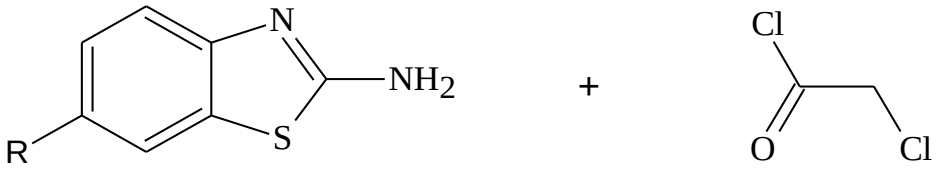


4-phenyl-2H-pyrimido[2,1-b][1,3]benzothiazole

1-(4-phenyl-4H-pyrimido[2,1-b][1, 3]benzothiazol-3-yl)ethanone

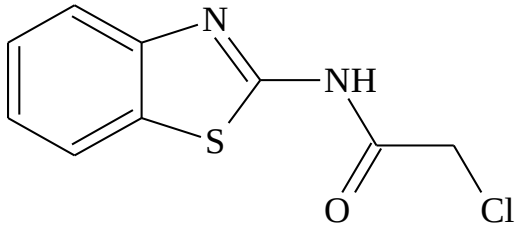
Reaction: 2

Synthesis of 5-Arylidene-2-imino-3-(benzo[d]thiazol-2-yl) thiazolidin-4-one derivative



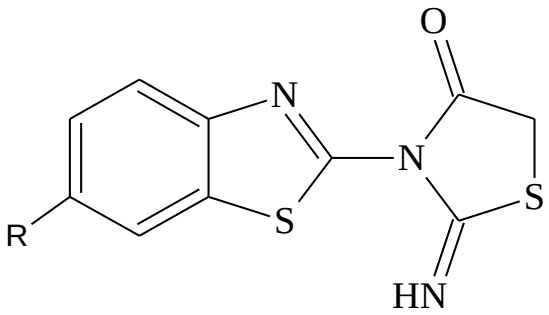
1,3-benzothiazol-2-amine chloroacetyl chloride

12 hrs
Reflux
Dry benzene



N-1,3-benzothiazol-2-yl-2-chloroacetamide

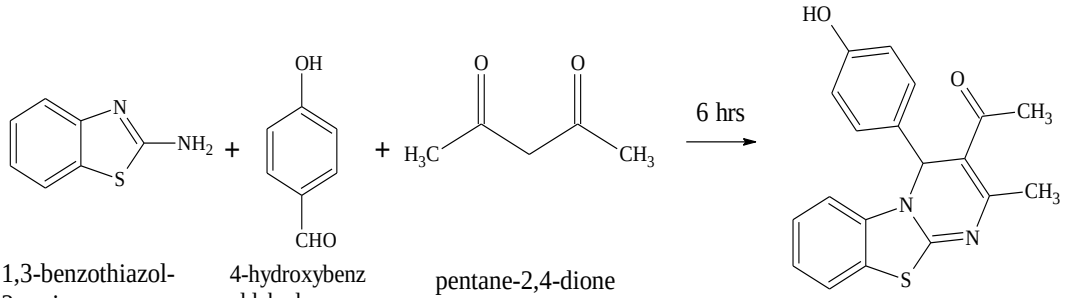
6 hrs
120^{0}C
NH_4SCN in Ethanol



3-(1,3-benzothiazol-2-yl)-2-imino-1,3-thiazolidin-4-one

SYNTHETIC REACTION

COMPOUND NAME: AH



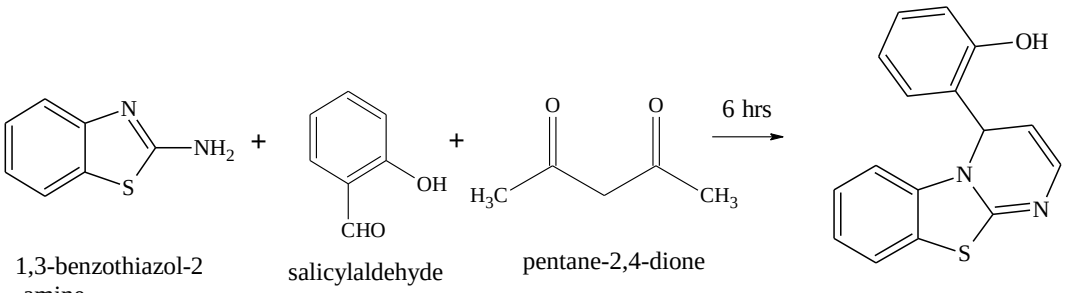
1,3-benzothiazol-2-amine

4-hydroxybenzaldehyde

pentane-2,4-dione

1-[4-(4-hydroxyphenyl)-2-methyl-4*H*-pyrimido[2,1-*b*][1,3]benzothiazol-3-yl] ethanone

COMPOUND NAME: DAA



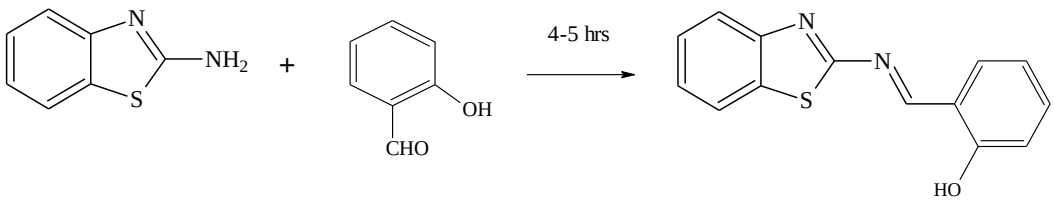
1,3-benzothiazol-2-amine

salicylaldehyde

pentane-2,4-dione

2-(4*H*-pyrimido[2,1-*b*][1,3]benzothiazol-4-yl)phenol

COMPOUND NAME: DAS

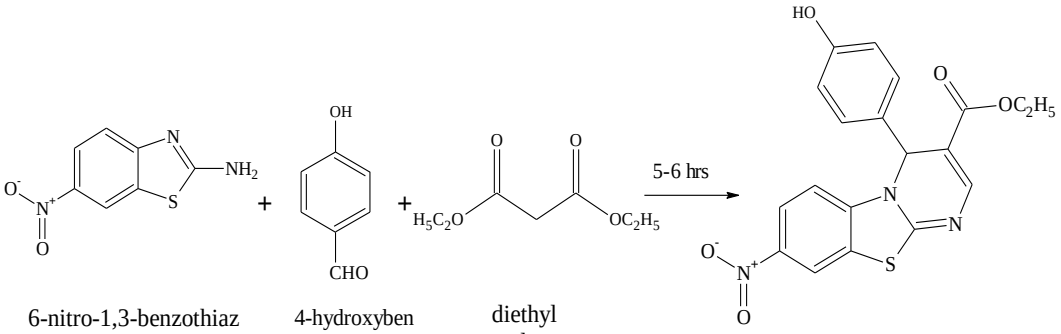


1,3-benzothiazol-2-amine

salicylaldehyde

2-[(E)-(1,3-benzothiazol-2-ylimino) methyl]phenol

COMPOUND NAME: DAC



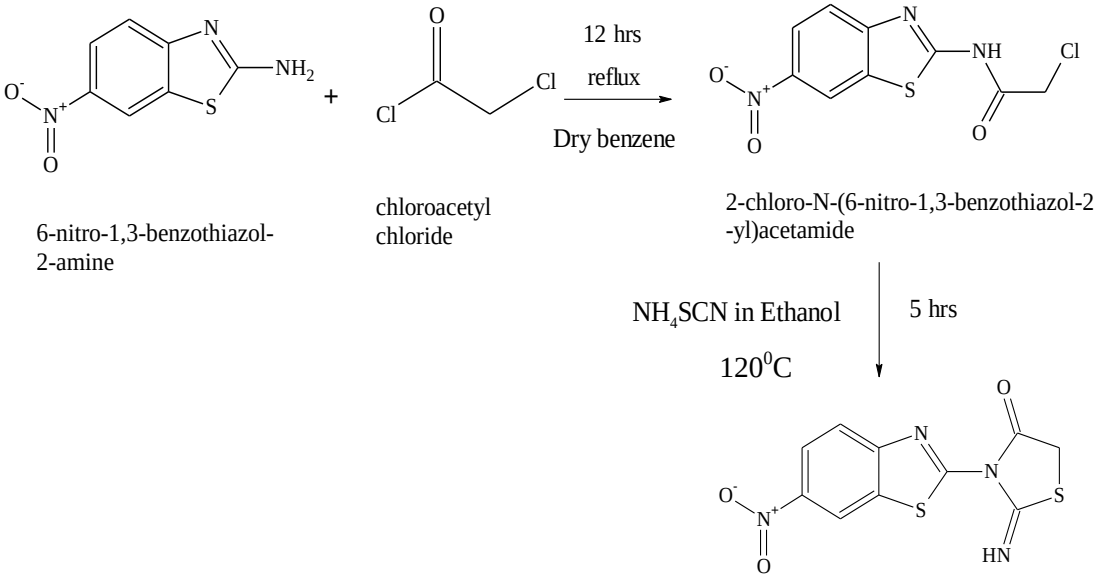
6-nitro-1,3-benzothiazol-2-amine

4-hydroxybenzaldehyde

diethyl malonate

ethyl 4-(4-hydroxyphenyl)-8-nitro-4H-pyrimido[2,1-b][1,3]benzothiazole-3-carboxylate

COMPOUND NAME: KS



6-nitro-1,3-benzothiazol-2-amine

chloroacetyl chloride

2-chloro-N-(6-nitro-1,3-benzothiazol-2-yl)acetamide

2-imino-3-(6-nitro-1,3-benzothiazol-2-yl)-1,3-thiazolidin-4-one

4.3 METHODS OF CHARACTERISATION

The synthesized compounds were identified by using the following methods.

Melting Point

The melting points of the compounds were determined by the capillary tube method.

Thin Layer Chromatography [77]

Pre-coated TLC plates with silica gel GF 250 were used. Samples of reactants and products were prepared with suitable solvents. Solvent system was prepared based on the nature of the compounds.

Stationary phase : Pre coated gel plate (silica gel GF 250)

Mobile phase : Ethyl acetate: Hexane (4:6) ; Pet ether : Ethyl acetate (1:4)

Visualizing agent : Iodine chamber.

The determination of the Rf value of the reactants and the final product was done.

The characterization was carried out using sophisticated methods like Infrared spectroscopy, Nuclear magnetic resonance spectroscopy and Mass spectroscopy.

Infra Red Absorption Spectroscopy[78]

IR (region 2.5-15μ) is a powerful tool for identifying the pure organic and inorganic compounds, with the exception of a few homo nuclear molecules such as O_2, N_2, Cl_2 all the molecular species absorb infrared radiation.

Instrument : FT-IR spectrophotometer (model no: 3000)

Sample technique: KBr Pellet technique.

NMR Spectroscopy [80]

Nuclear magnetic resonance involves the interaction between oscillating magnetic field of electromagnetic radiation and the magnetic energy of the hydrogen nucleus or some other type of nuclei when these are placed in an external static magnetic field.

NMR enables us to study the number of equivalent protons and their electronic environment. It reveals the different chemical environment in which the proton is present and helps us to ascertain the structure of molecule.

The number of signals in an NMR spectrum denotes the number of the set of equivalent protons in a molecule. The position of the signals in the spectrum helps us to know the nature of protons such as aromatic, aliphatic, acetylenic, vinyl, adjacent to some electron attracting or electron-releasing group etc.

Instrument used : BRUKER Advance 500 NMR spectrometer
Solvent : Deuterated Dimethyl Sulphoxide
Internal standard : Tetramethylsilane(TMS).

Mass Spectroscopy[77,80]

Mass spectroscopy is an analytical technique used to establish the molecular weight and help in the determination molecular structure of the analyte under investigation. In this technique, the compound under investigation is bombarded with a beam of electrons producing ionic fragments of the original species. The relative abundance of the fragment ion formed depends on the stability of the ion and of the lost radical. The resulting charged particles are then separated according to their masses. Each kind of ion has a particular ratio of mass to charge, *i.e.* m/z ratio (value). Mass spectrum is a record of information regarding various masses produced and their relative abundances.

LC-MS: Liquid chromatography with mass spectroscopy is used to characterize the non-volatile compounds but GC-MS is used to characterize only the volatile compounds.

Q-Tof-Mass Spectroscopy (Q-Tof micro hybrid quadrupole Time of flight mass spectrometer) with electro spray ionization and in JEOL GCMATE II GC-MS.

4.4 BIOLOGICAL EVALUATION

The designed and synthesized molecules need to be screened for their activity to inhibit the growth of the Mycobacterium tuberculosis.

ANTITUBERCULAR ACTIVITY[86]

The following methods are generally used for *in vitro* studies for the evaluation of anti-tubercular activity.

- Resazurin Micro plate Assay (REMA)
- Nitrate Reductase Assay (NRA)
- Micro plate Alamar Blue Assay (MABA)
- 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT)
- Middle Brook 7H11 Agar dilution Assay
- Broth Micro dilution Method
- BACTEC system
- Luciferase Reporter Phage Assay

The synthesized compounds may be evaluated for anti-tubercular activity using any of the above methods. In this technique, Microplate Alamar Blue Assay is used to evaluate the anti-tubercular activity. It is an easy and economic method.

MICROPLATE ALAMAR BLUE ASSAY [88]

Alamar blue dye is used as an indicator for the determination of viable cells.

The oxidized form, Resazurine (also called as diazo-resorcinol, azoresorcin, resazoin and resazurine) is non- toxic, non-fluorescent and blue in colour which becomes pink and fluorescent upon reduction to resorufin by viable cells.

Growth is measured qualitatively by a visual colour change and the amount of fluorescence produced is proportional to the number of the living cells, which is determined by colorimetric, and fluorimetric methods.

Advantages

- ✓ High sensitivity
- ✓ Does not require cell lysis
- ✓ Works well with different types of cells
- ✓ Safe and economical
- ✓ Results are linear and quantitative
- ✓ Water soluble
- ✓ Not required cell proliferation assays

Assay Procedure For Estimating Anti-Tb Activity Using[89]

⇒ The anti-mycobacterial activity of compounds is assessed against M. tuberculosis using Alamar Blue micro plate assay (MABA).

⇒ This methodology is non-toxic, uses a thermally stable reagent and shows good correlation with proportional and BACTEC radiometric method.

⇒ Briefly, 200 micro litter of sterile de-ionized water is added to all outer perimeter wells of sterile 96 wells plate to minimize evaporation of medium in the test wells during incubation.

⇒ The 96 wells plate receives 100 micro litter of the middle brook 7H9 broth and serial dilution of compounds is made directly on plate.

⇒ The final drug concentrations tested are 100 to 0.2 micro gram/ml.

⇒ Plates are covered and sealed with paraffin and incubated at 37^0C for five days.

⇒ After this time, 25 micro litters of freshly prepared 1:1 mixture of Alamar blue reagent and 10% tween 80 is added to the plate and incubated for 24 hrs.

⇒ A blue colour in the well is interpreted as no bacterial growth, and pink colour was scored as growth.

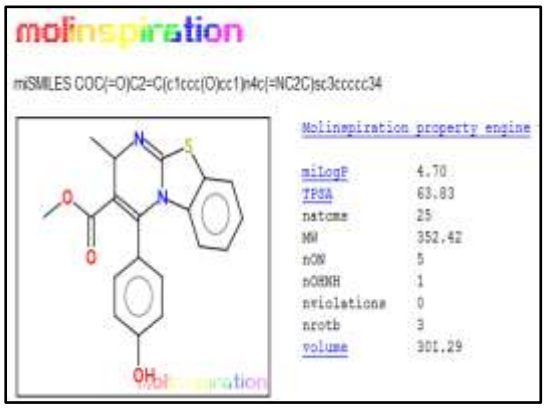
⇒ The MIC is defined as lowest drug concentration, which prevents the colour change from blue to pink.

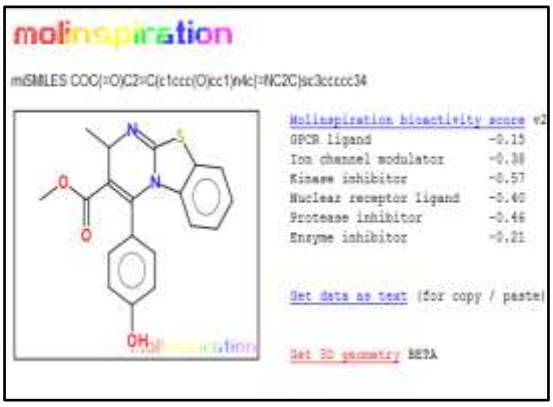
RESULT AND DISCUSSION

5.1 INSILICO SCREENING OF DRUG LIKENESS

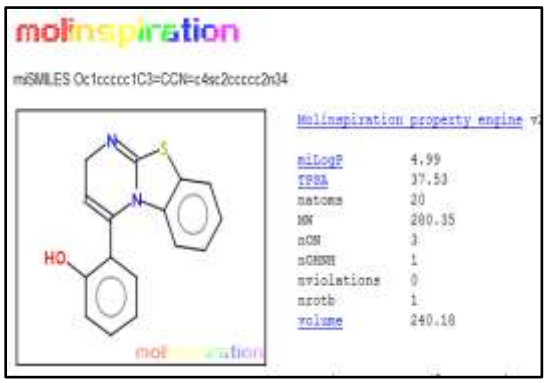
MOLINSPIRATION® Cheminformatics software used to evaluate drug likeness.

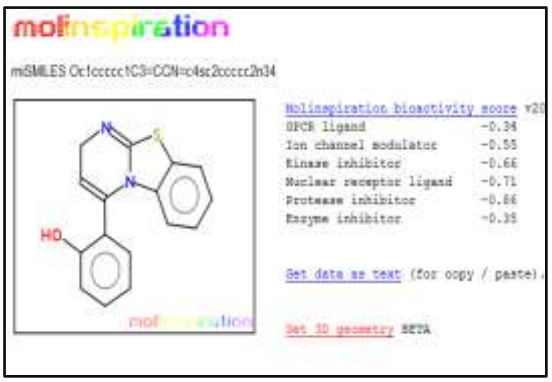
Sample code : AH



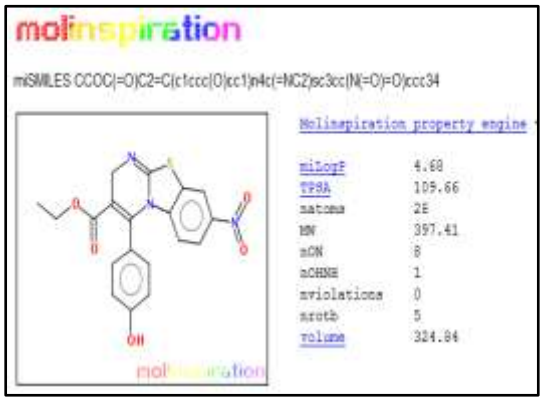


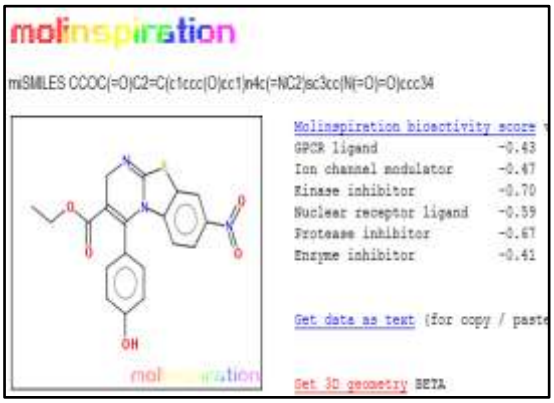
Sample code : DAA



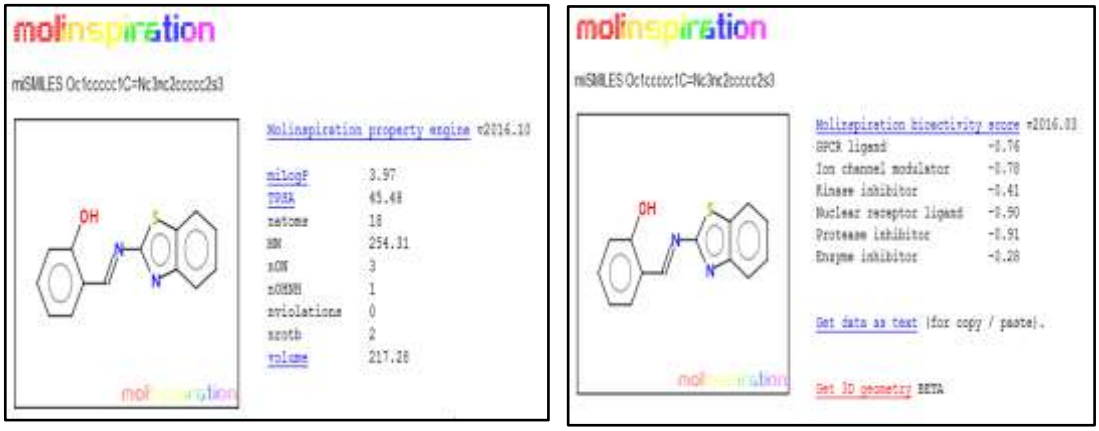


Sample code : DAC





Sample code : DAS



Sample code : KS

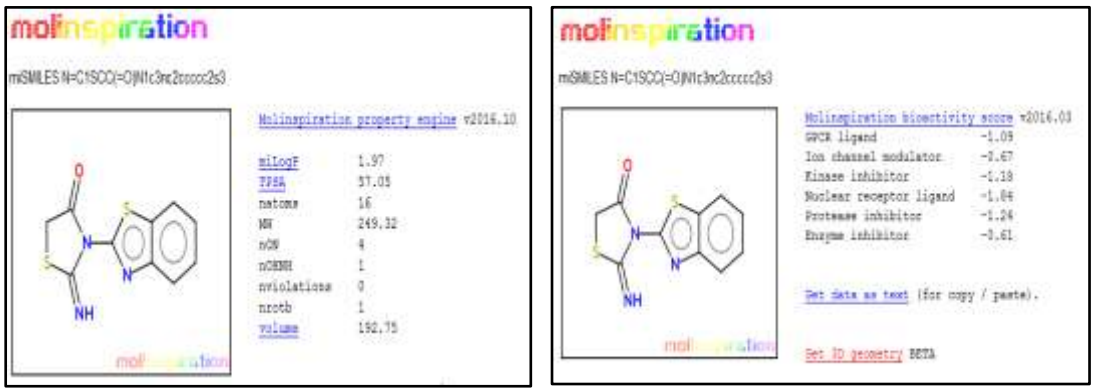


Figure 7: Calculation of Molecular Properties and Biological Activities

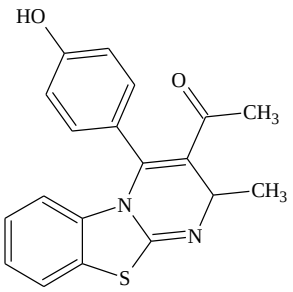
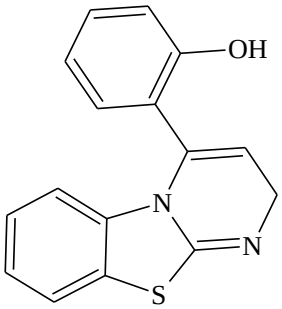
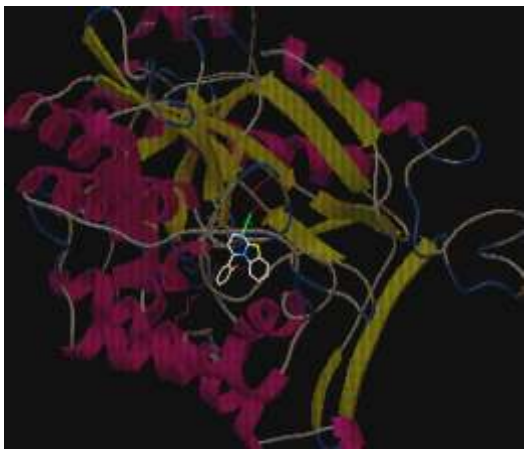
The Lipinski's Rule of Five describes molecular properties important for a drug's pharmacokinetics in the human body, including their absorption, distribution, metabolism and excretion (ADME).

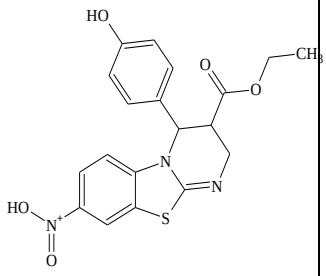
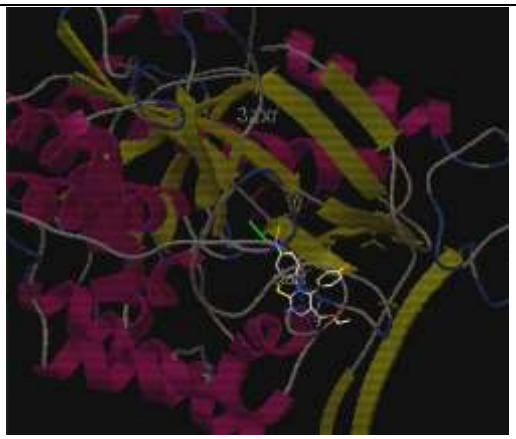
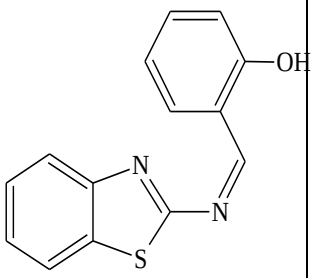
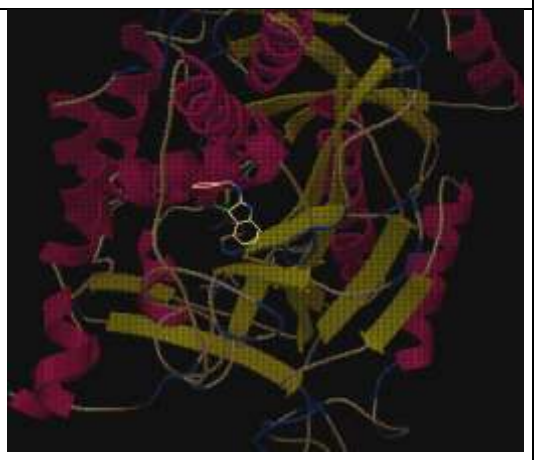
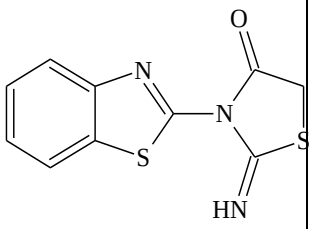
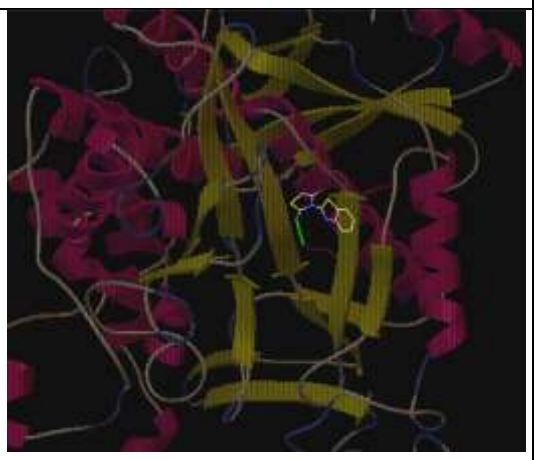
These 5 compound shows no violations and obey the Lipinski's Rule of Five that is confirmed by using MOLINSPIRATION® Cheminformatics software. The above 5 scaffolds were used for the synthesis and for further studies.

DOCKING

The derivatives of the selected 5 scaffolds were docked against Glutamine Synthetase 1(3ZXR -Mtb enzyme). The molecules were screened for good docking score and interactions by using Autodock® software.

Table 2: The molecules with good docking score were mentioned as below:

Code name	Structures	Docking score Kcal/mol	Docking view
AH		**-7.51**	
DAA		**-7.77**	

Code name	Structures	Docking score Kcal/mol	Docking view
DAC		**-8.45**	
DAS		**-6.67**	
KS		**-6.41**	

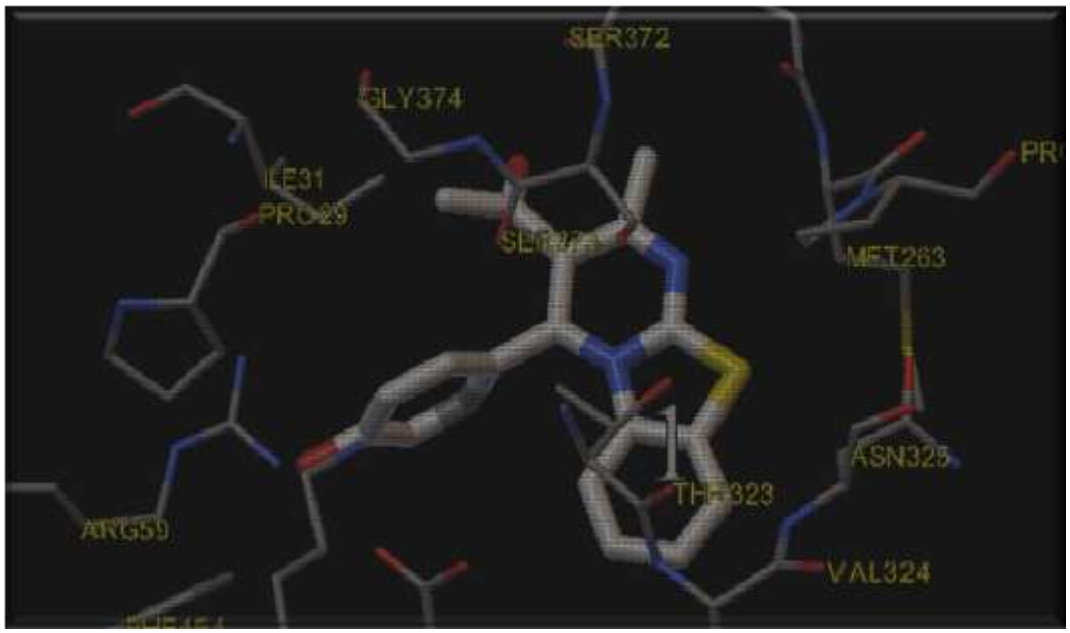


Fig 8: Interaction View Of AH

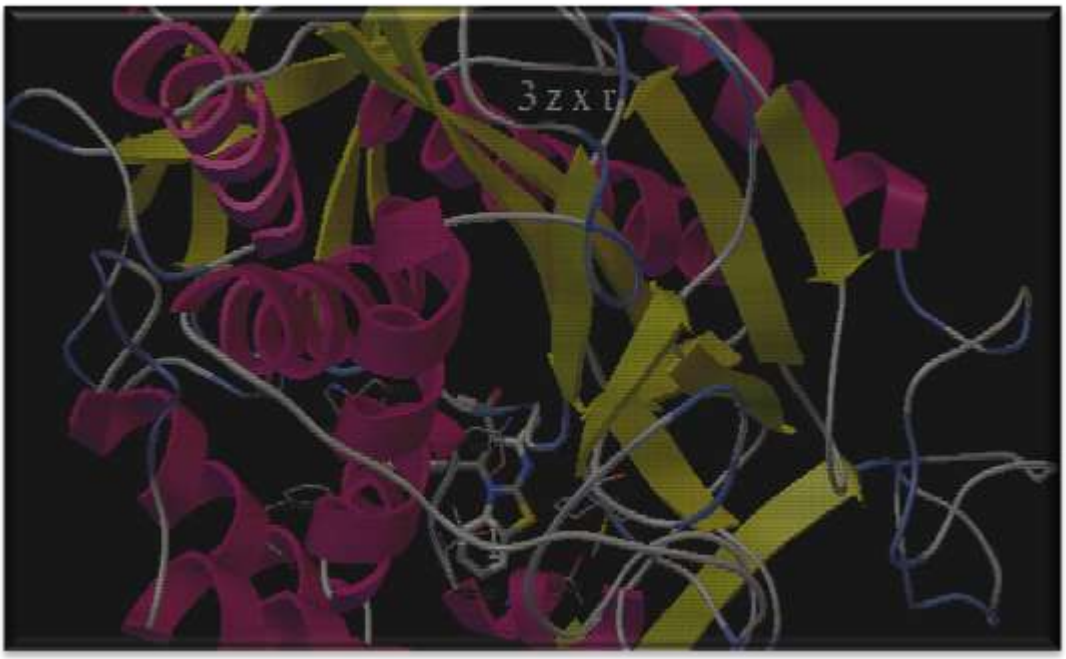


Fig 9: Docking View Of AH

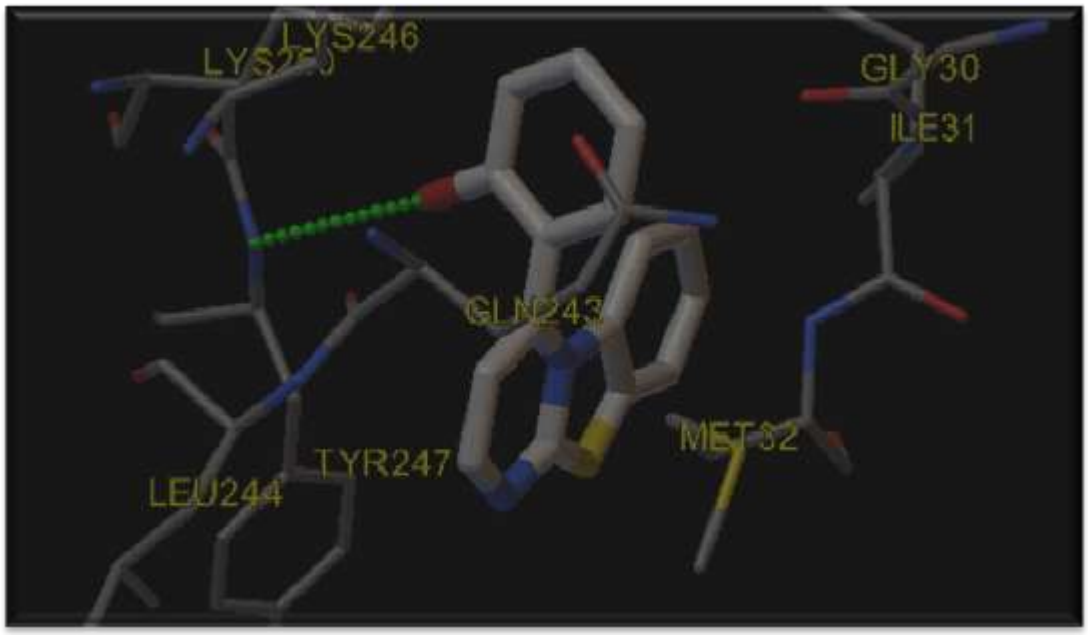


Fig 10: Interaction View of DAA

Green colour bond indicates the Hydrogen bond interaction

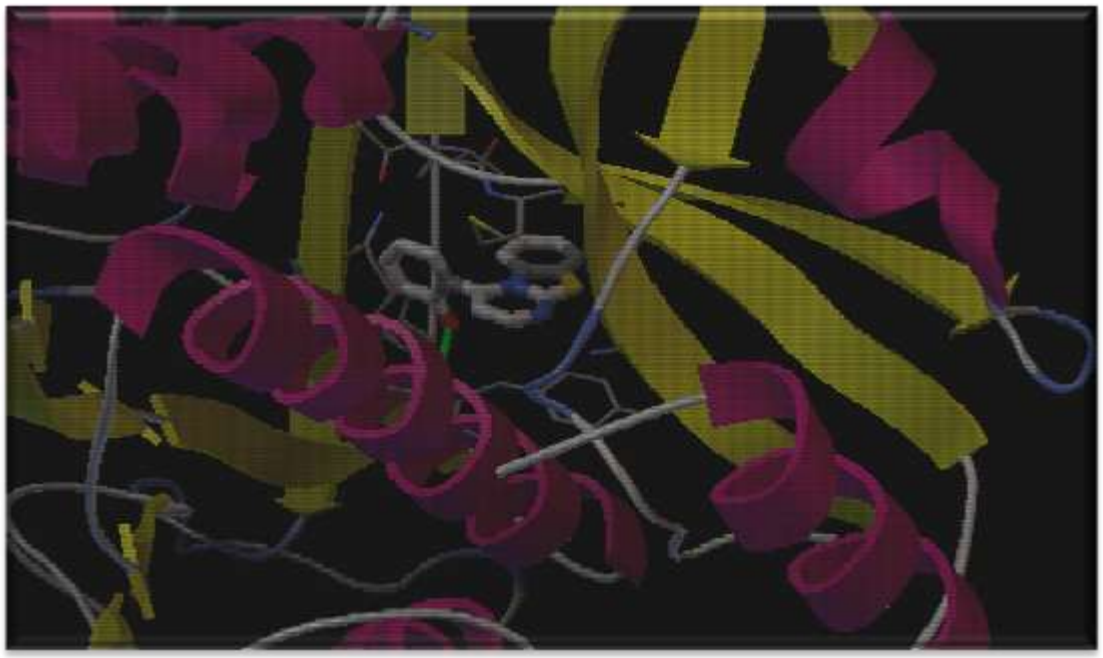


Fig 11: Docking View of DAA

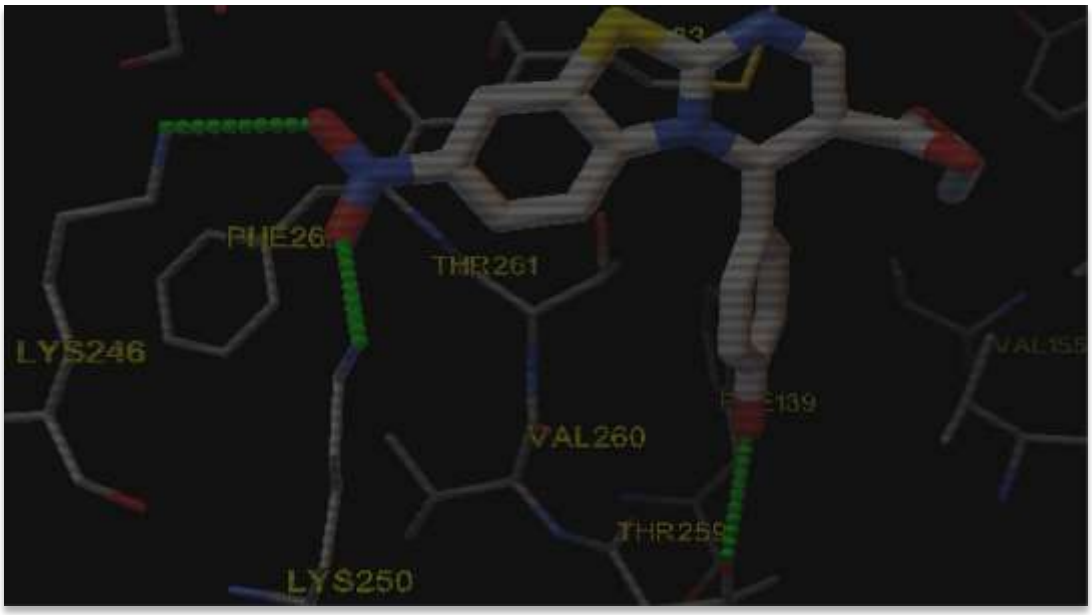


Fig 12: Interaction View of DAC

Green colour bond indicates the hydrogen bond interaction

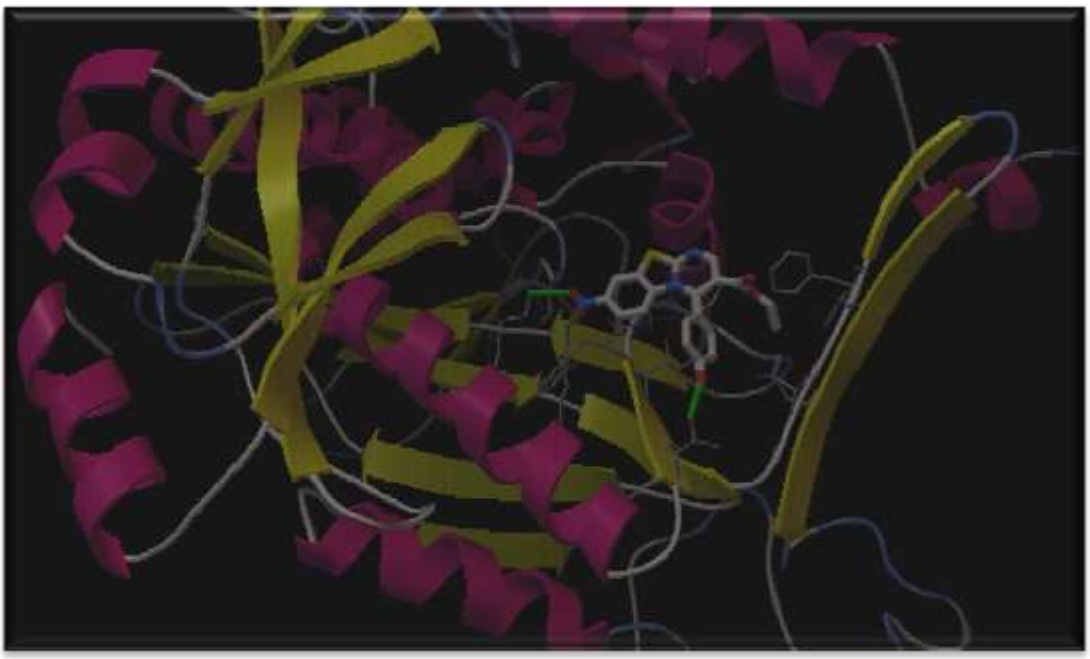


Fig 13: Docking View of DAC

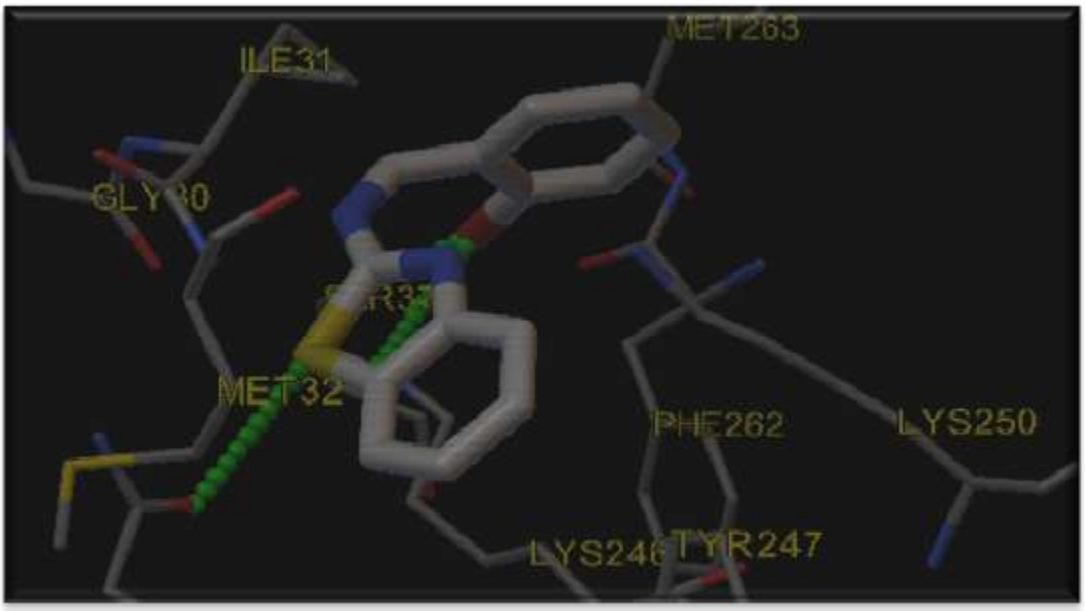


Fig 14: Interaction View of DAS

Green colour bond indicates the hydrogen bond interaction

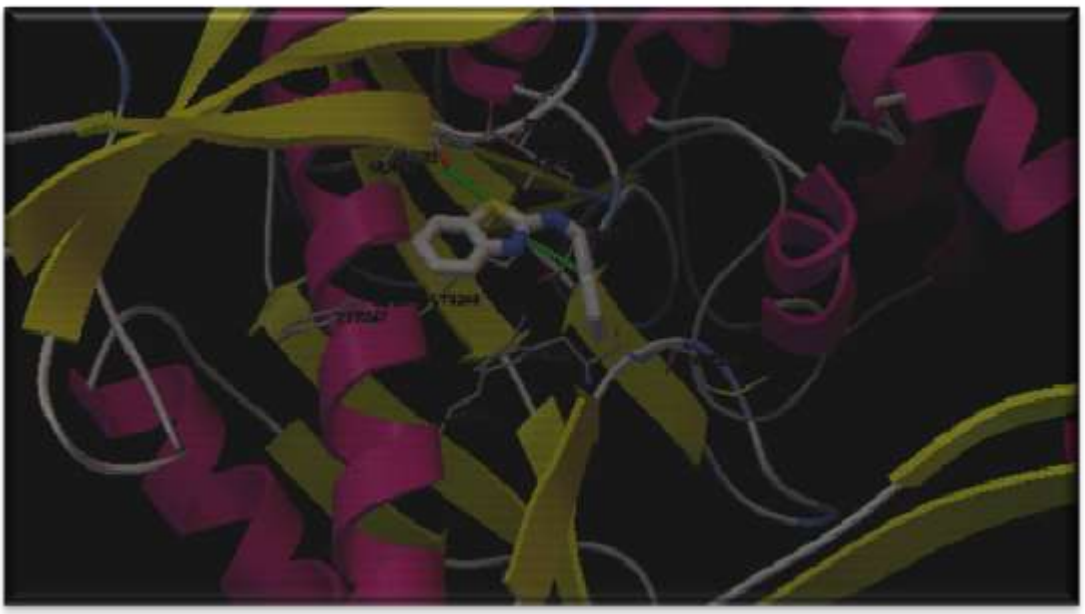


Fig 15: Docking View of DAS

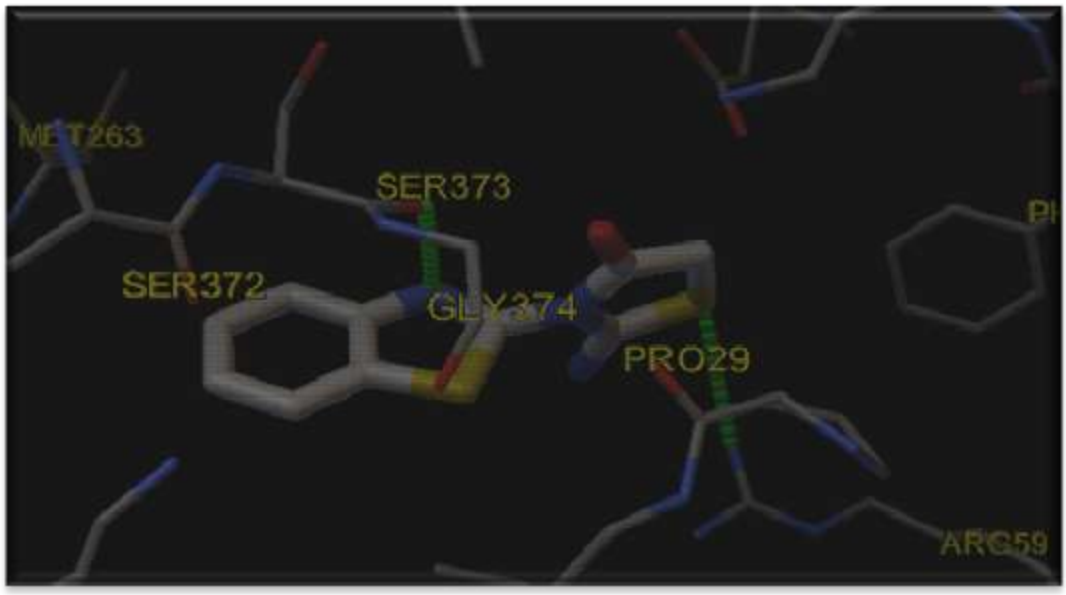


Fig 16: Interaction View of KS

Green colour bond indicates the hydrogen bond interaction

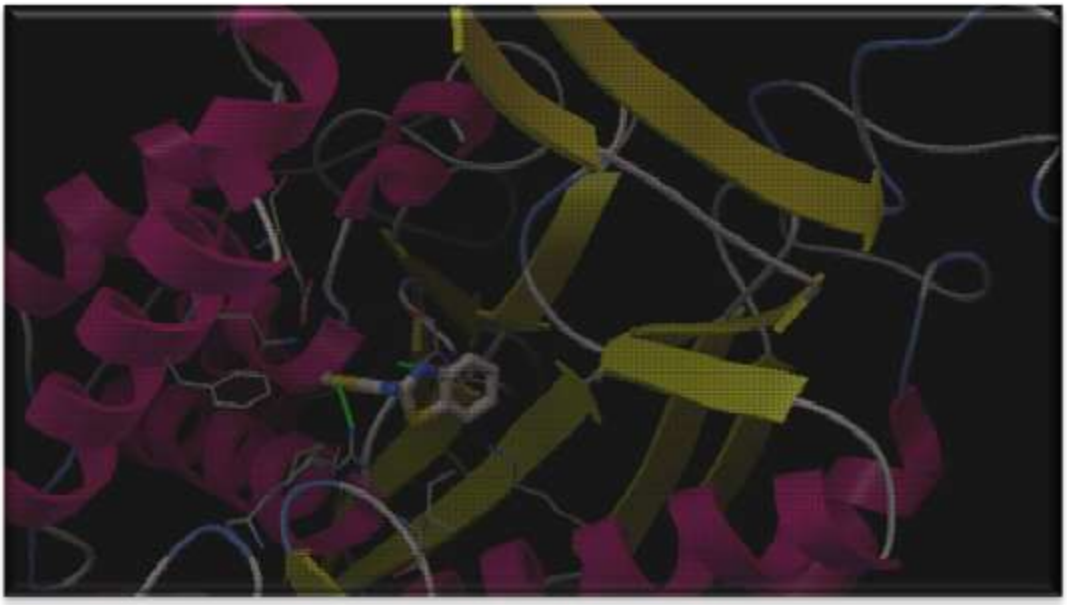
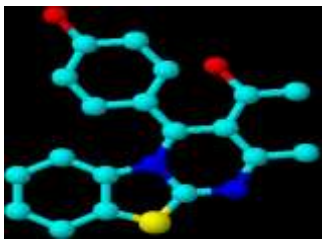
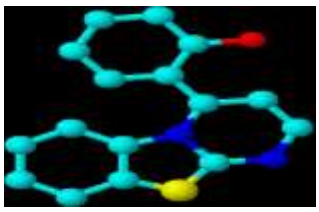
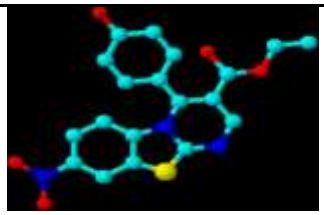
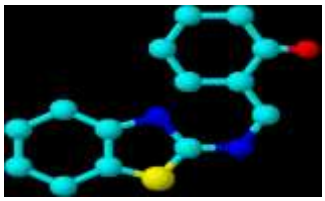
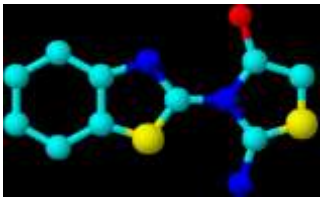
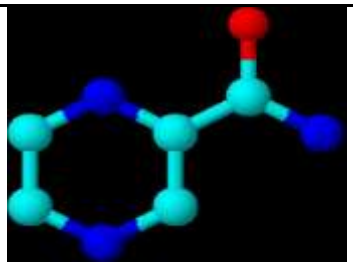


Fig 17: Docking View of KS

Table 3: Interaction with Amino acids

Name	Structure	Docking score	Interaction with amino acid
AH		-7.51	ASN 325,ARG 59, GLY 374, GLU 459,ILE 31,LYS 455,MET 263, PHE -262,454, SER-372,373, PRO -29,264 THR 323, VAL 324.
DAA		-7.71	H- bond: ARG 24, TYR 247; GLN 243, GLY 30, ILE 31, MET 32, LEU 244, LYS- 246, 250, TYR 247.
DAC		-8.45	H-bond: LYS 246, LYS 250, TYR 259; MET 263, PHE 262, SER 372, TYR- 153,261.
DAS		-6.67	H- bond: GLY 374, PRO 29, SER 373;ARG 59, GLY 30, ILE 31, LYS 246, MET 263, PHE 262.
KS		-6.41	ARG 24, ASN 251, GLN 243, HIS 34, GLY 30, ILE 31, LYS 246, MET 32, TYR 247.
Pyrazinamide		-4.41	H-bond: ARG 24; ARG 79, ASN -24, 90, 92, GLN 243, LEU 244, MET 32, THR 77, TYR 247.

IN-SILICO TOXICITY PREDICTION

Insilico toxicity prediction was done for the filtered 5 compounds using OSIRIS® Property Explorer. This software is available for access in the Organic Chemistry Portal. Using the prediction tool, mutagenicity, tumorogenicity, skin irritation and reproductive effects were calculated. The results were colour-coded. The green colour represents that the compounds are non-toxic. Yellow color indicates moderate and red colour indicates severe toxicity of the chemicals respectively.

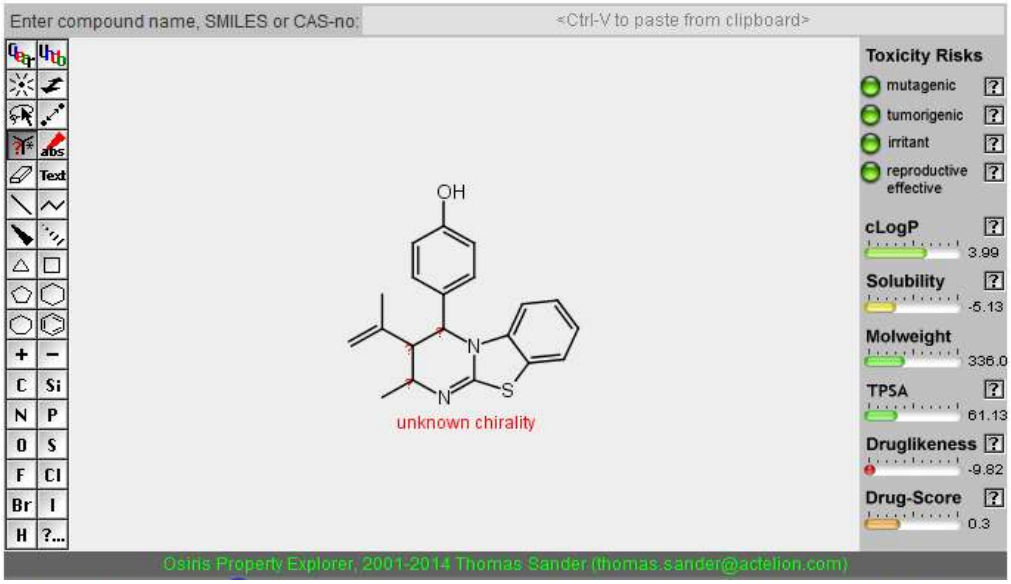


Figure 18: Toxicity Prediction of AH

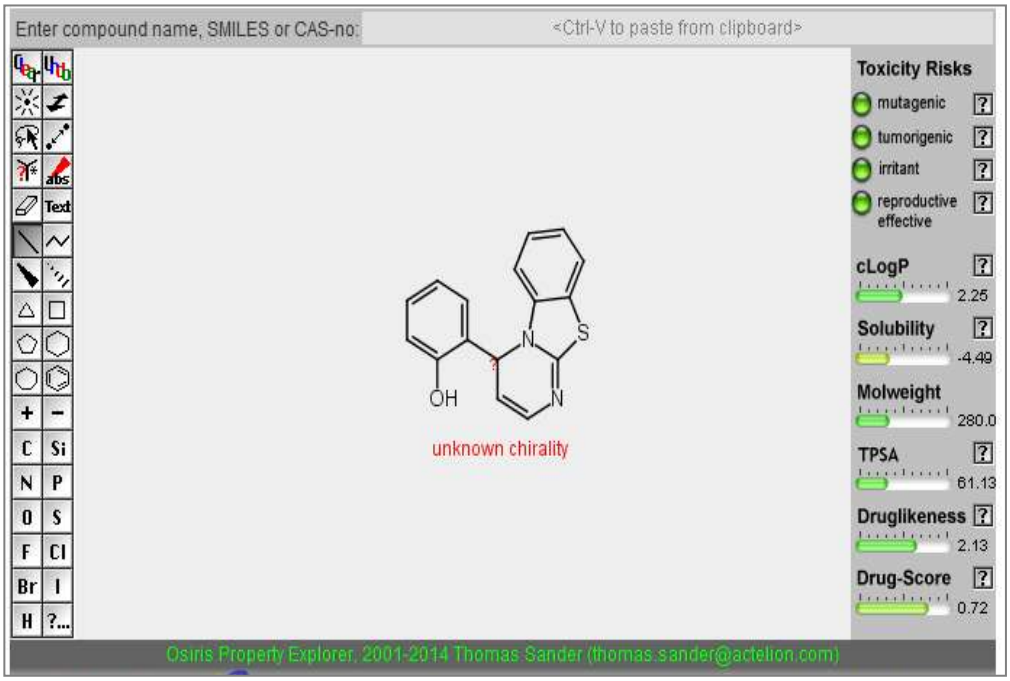


Figure 19: Toxicity Prediction of DAA

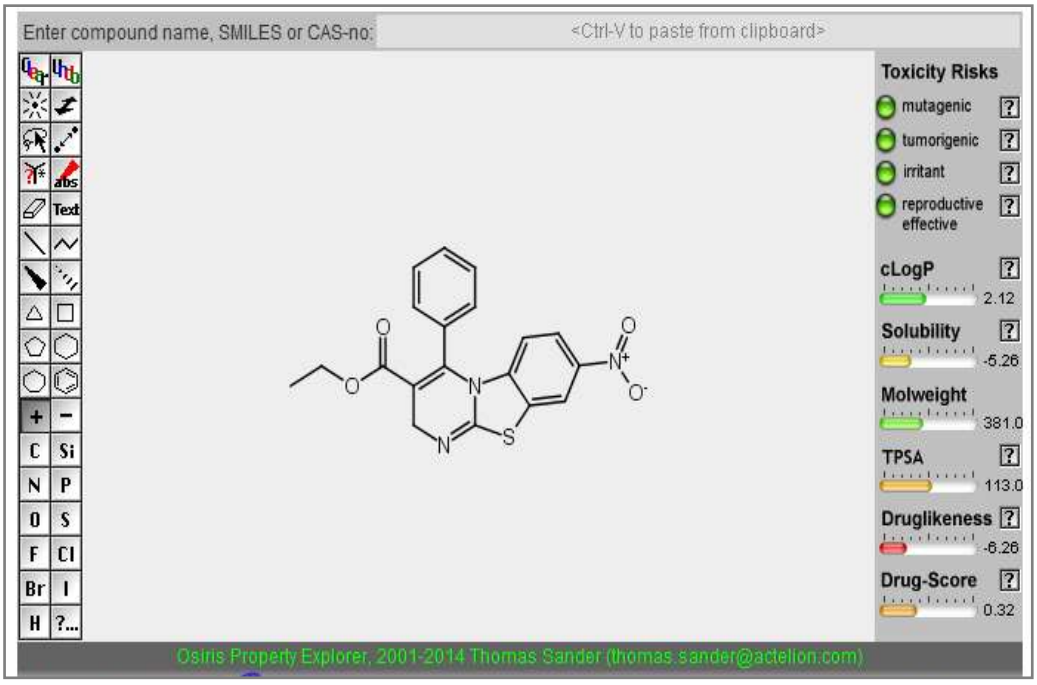


Figure 20: Toxicity Prediction of DAC

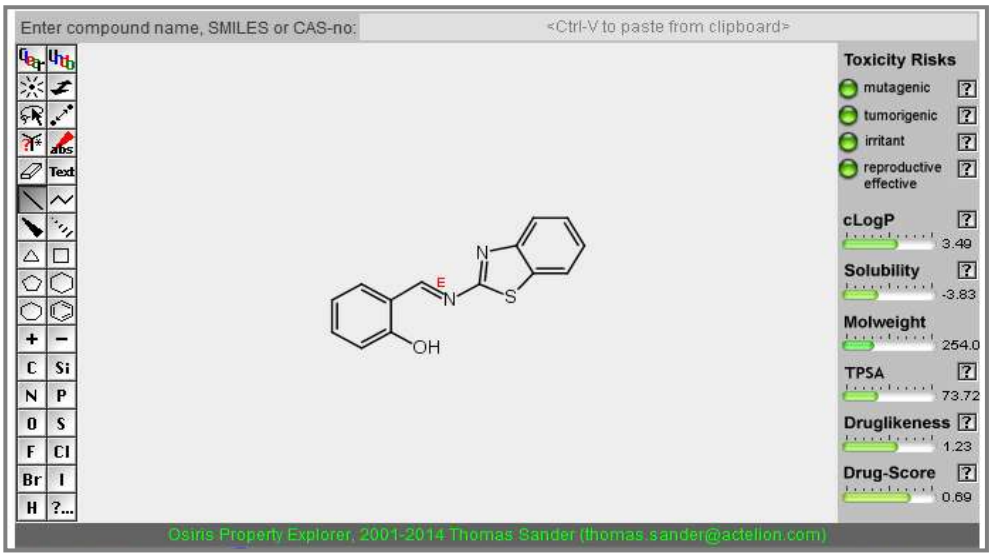


Figure 21: Toxicity Prediction of DAS

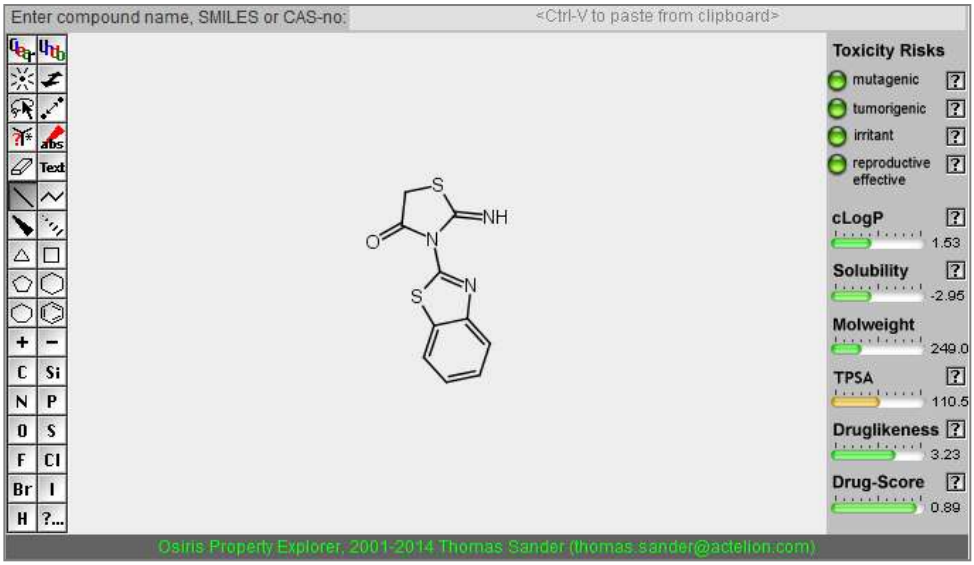


Figure 22: Toxicity Prediction of KS

The selected 5 compounds are non toxic which was confirmed by the Osiris® property explorer software.

5.2 PRODUCT PROFILE

The selected 5 scaffolds were synthesized in an appropriate manner and recrystallized. The synthesized compounds were evaluated for their purity through melting point determination and checking for absence of parent compounds or other new compounds by TLC.

The Rf value of parent compounds

2 amino benzothiazole - 0.75

Salicyaldehyde - 0.54

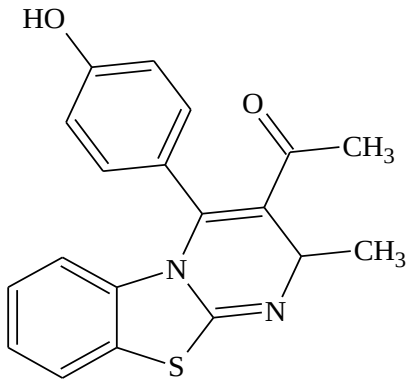
4-OH benzaldehyde - 0.59

Table 4: The synthesized compounds were evaluated by the following:

S.No	CODE NAME	MOLECULAR WEIGHT	YIELD %	MELTING POINT	Rf VALUE
1.	AH	336.4	67%	198-200^{0}C	0.68
2.	DAA	280.3	59%	115-117^{0}C	0.85
3.	DAC	400.4	74%	198-200^{0}C	0.68
4.	DAS	254.3	69%	118-120^{0}C	0.86
5.	KS	249.3	70%	198-200^{0}C	0.63

The Rf value of the synthesized compounds was varied from the Rf value of the reactants. It is concluded that the reaction was completed. The compounds were obtained in acceptable yield. The melting point range confirmed the purity of the product.

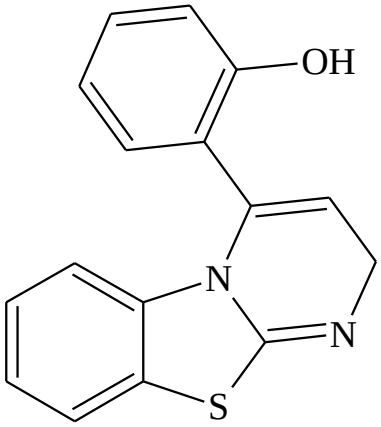
Code: **AH**



Methyl4-(4-hydroxyphenyl)-2-methyl-2H-pyrimido[2,1b][1,3]benzothiazole-3-carboxylate

Molecular formula	$C_{19}H_{16}N_2O_3S$
Molecular weight	336.41
Appearance	Dark green colour
Melting point	198-200^{0}C
Composition	C(67.84%), H(4.79%), N(8.33%), O(9.51%), S(9.53%)
Molar refractivity	96.06 ±0.5 cm^3
Molar volume	247.1 ±7.0 cm^3
Surface tension	53.4 ±7.0 dyne/cm
Density	1.36 ±0.1 g/cm^3
Parachor	668.4 ±8.0 cm^3
Index of refraction	1.704 ±0.05
Polarizabilty	38.08 ±0.5 10^{-24} cm^3

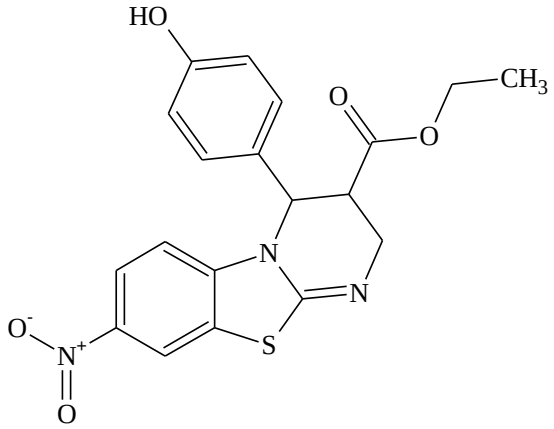
Code: **DAA**



2-(2H-pyrimido [2,1-b][1,3]benzothiazol-4-yl)phenol

Molecular formula	$C_{16}H_{12}N_2OS$
Molecular weight	280.345
Appearance	Dark green
Melting point	115-117^{0}C
Composition	C (68.55%), H (4.31%), N(9.99%), O (5.71%), S (11.44%)
Molar refractivity	81.75 ±0.5 cm^3
Molar volume	203.4 ±7.0 cm^3
Surface tension	58.2 ±7.0 dyne/cm
Density	1.37 ±0.1 g/cm^3
Parachor	561.9 ±8.0 cm^3
Index of refraction	1.736 ±0.05
Polarizabilty	32.41 ±0.5 10^{-24} cm^3

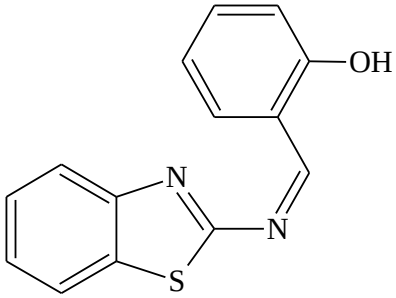
Code: **DAC**



Ethyl 4-(4-hydroxyphenyl)-8-nitro-2H-pyrimido [2, 1-b][1,3]benzothiazole-3-carboxylate

Molecular formula	$C_{19}H_{18}N_3O_5S$
Molecular weight	400.429
Appearance	Yellow
Melting point	198-200^{0}C
Composition	C(56.99%), H(4.53%), N(10.49%), O(19.98%), S(8.01%)
Molar refractivity	103.30 ±0.5 cm^3
Molar volume	259.8 ±7.0 cm^3
Surface tension	66.0 ±7.0 dyne/cm
Density	1.52 ±0.1 g/cm^3
Parachor	740.5 ±8.0 cm^3
Index of refraction	1.726 ±0.05
Polarizabilty	40.95 ±0.5 10^{-24} cm^3

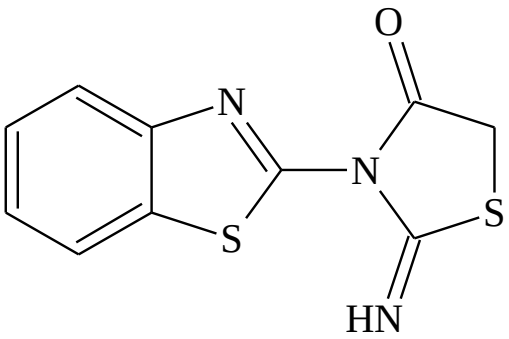
Code: **DAS**



2-[(Z)-(1,3-benzothiazol-2-ylimino)methyl]phenol

Molecular formula	$C_{14}H_{10}N_2OS$
Molecular weigh	254.308
Appearance	Pale yellow
Melting point	118-120 ^{0}C
Composition	C (66.12%), H(3.96%), N(11.02%), O (6.29%), S (12.61%)
Molar refractivity	74.18 ±0.5 cm^3
Molar volume	193.6 ±7.0 cm^3
Surface tension	54.0 ±7.0 dyne/cm
Density	1.31 ±0.1 g/cm^3
Parachor	525.1 ±8.0 cm^3
Index of refractio	1.691 ±0.05
Polarizabilty	29.41 ±0.5 10^{-24} cm^3

Code: **KS**



3-(1,3-benzothiazol-2-yl)-2-imino-1,3-thiazolidin-4-one

Molecular formula	$C_{10}H_7N_3OS_2$
Molecular weight	249.314
Appearance	Pale yellow
Melting point	198-200^{0}C
Composition	C (48.17%), H (2.83%),N(16.85%), O (6.42%), S (25.72%)
Molar refractivity	66.37 ±0.5 cm^3
Molar volume	146.6 ±7.0 cm^3
Surface tension	82.3 ±7.0 dyne/cm
Density	1.69 ±0.1 g/cm^3
Parachor	441.7 ±8.0 cm^3
Index of refraction	1.865 ±0.05
Polarizabilty	26.31 ±0.5 10^{-24} cm^3

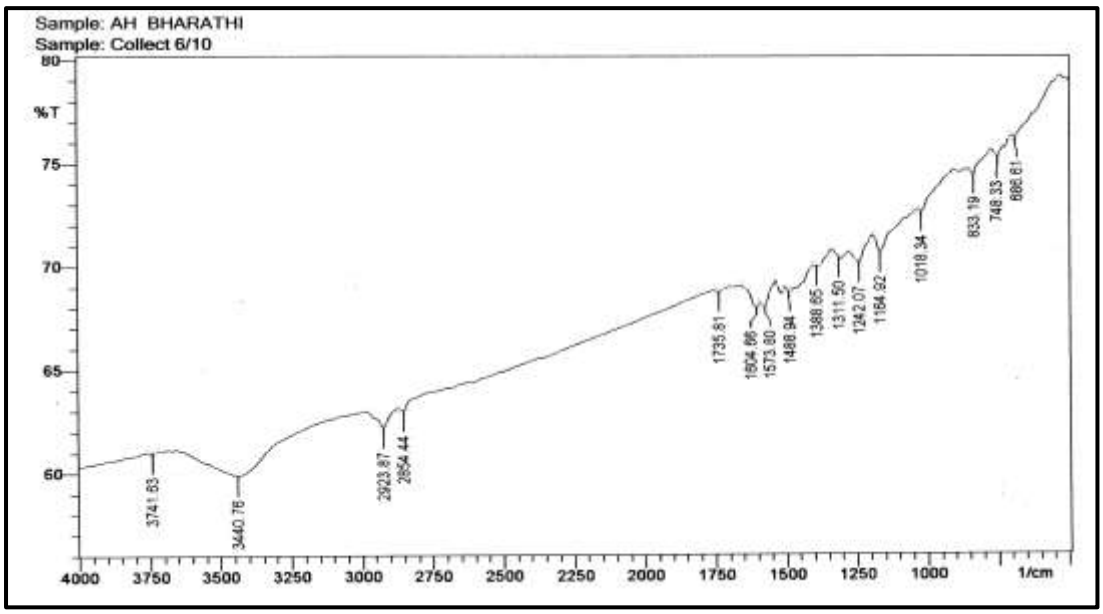
5.3 CHARACTERIZATION

Infrared Spectroscopy

The samples were prepared by the KBr pellet techniques and the samples are analyzed by FT-IR technique.

Sample code: AH

.

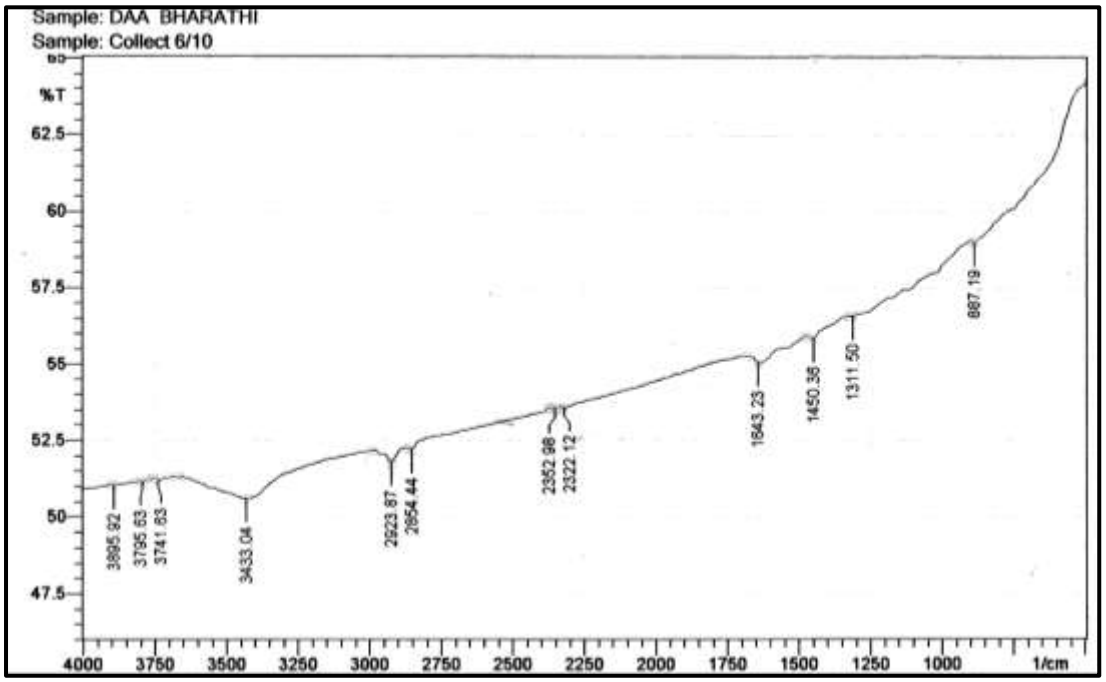


IR spectrum of AH

Table 5: IR Interpretation of AH

S.NO	WAVE NUMBER	TYPES OF VIBRATIONS	FUNCTIONAL GROUP
1.	3440 cm^{-1}	OH Stretching	Aromatic OH groups
2.	2923 cm^{-1}	CH Stretching	Alkyl group
3.	1735 cm^{-1}	C=O Stretching	Ketone group
4.	1488 cm^{-1}	C=N bending	Heteroaromatic compound(in Ring)
5.	1242 cm^{-1}	NH bending	Secondary amino group

Sample Code: DAA

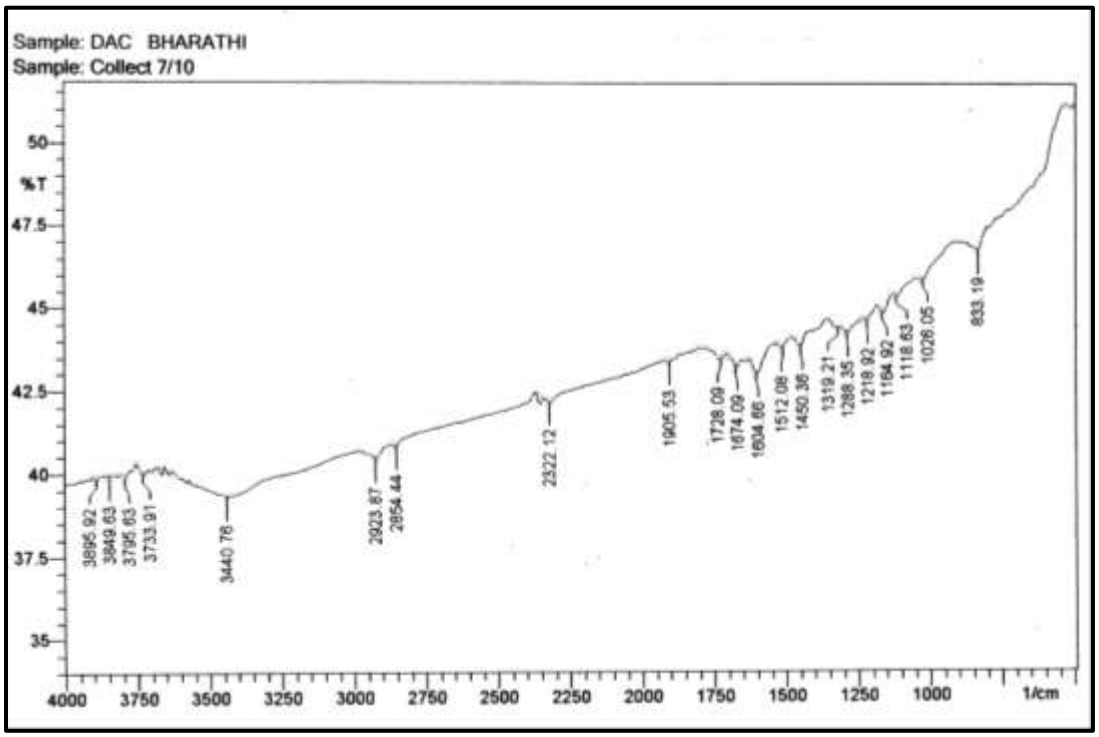


IR spectrum of DAA

Table 6: IR Interpretation of DAA

S.No	WAVE NUMBER	TYPES OF VIBRATIONS	FUNCTIONAL GROUP
1.	3433 cm^{-1}	OH – Stretching	Aromatic OH Groups
2.	2923 cm^{-1}	CH- Stretching	Alkyl Group
3.	2352 cm^{-1}	NH -Stretching	Secondary Amino Group
4.	1643 cm^{-1}	C=C Stretching	Aromatic Ring
5.	1450 cm^{-1}	C=N Bending	Heteroaromatic Compound(In Ring)

Sample code: DAC

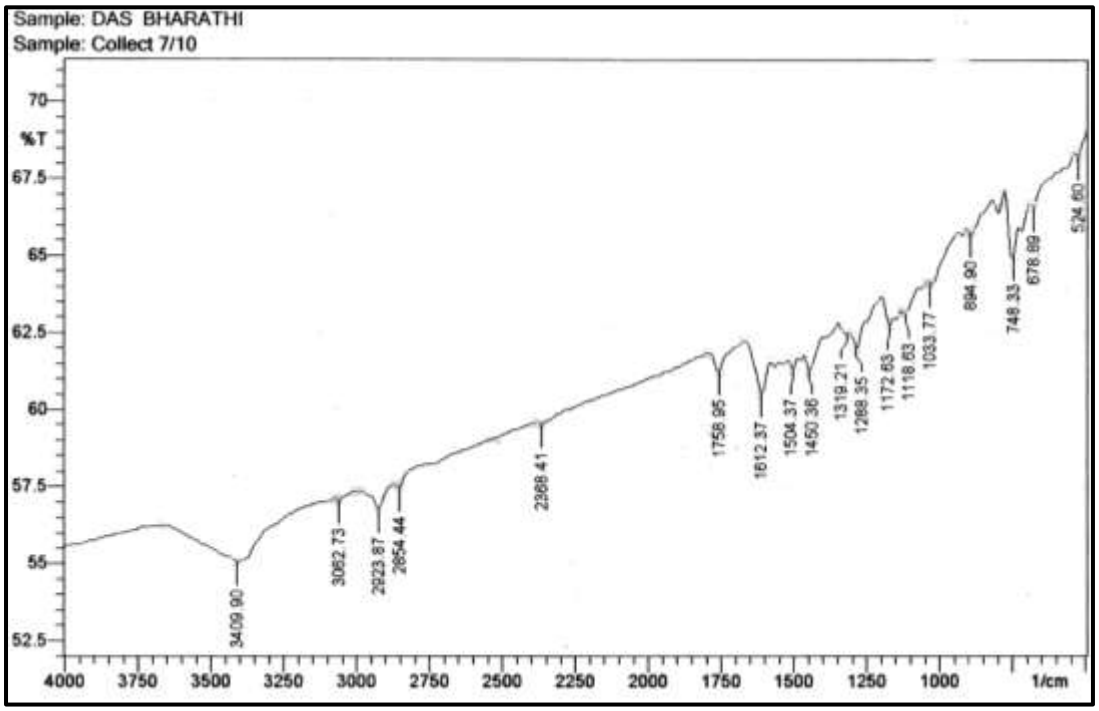


IR spectrum of DAC

Table 7: IR Interpretation of DAC

S.No	WAVE NUMBER	TYPES OF VIBRATION	FUNCTIONAL GROUPS
1.	3440 cm^{-1}	OH– Stretching	Aromatic OH Groups
2.	2854 cm^{-1}	CH- Stretching	Alkyl Group
3.	2322 cm^{-1}	NH -Stretching	Secondary Amino Group
4.	1728 cm^{-1}	C=O Stretching	Aliphatic Ester Group
5.	1512 cm^{-1}	NO_2 Stretching	Aromatic Nitro Group
6.	1450 cm^{-1}	C=N Bending	Heteroaromatic Compound(In Ring)

Sample code: DAS

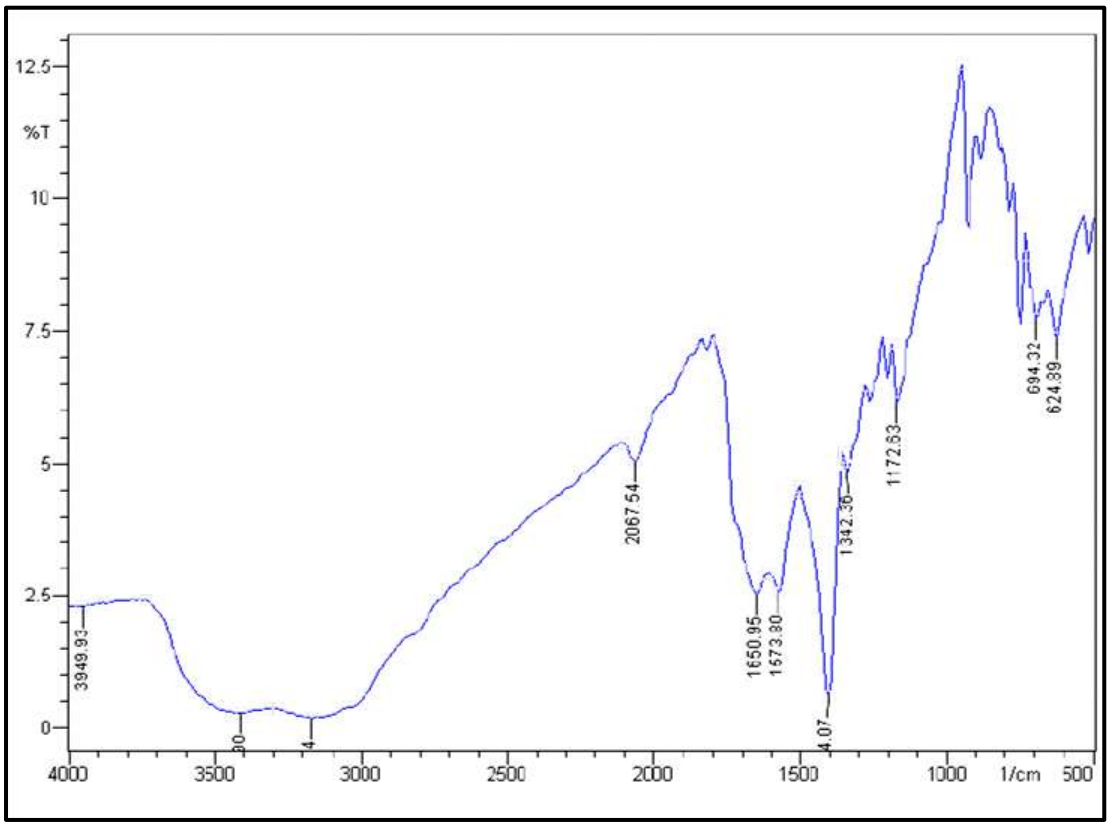


IR spectrum of DAS

Table 8: IR Interpretation of DAS

S.No	WAVE NUMBER	TYPES OF VIBRATION	FUNCTIONAL GROUP
1.	3409 cm^{-1}	OH– Stretching	Aromatic OH Group
2.	3062 cm^{-1}	CH- Stretching	Aromatic Group
3.	2368 cm^{-1}	C=C -Stretching	Aromatic Ring
4.	1450 cm^{-1}	C=N Stretching	Heteroaromatic Compound 2(In Ring)
5.	1319 cm^{-1}	C=N Stretching	Aliphatic Amino Group

Sample code: KS



IR spectrum of KS

Table 9: IR Interpretation of KS

S.No	WAVE NUMBER	TYPES OF VIBRATION	FUNCTIONAL GROUP
1.	3170 cm^{-1}	CH Stretching	Aromatic Group
2.	2067 cm^{-1}	NH Stretching	Amino Group
3.	1650 cm^{-1}	C=O-Stretching	Ketone Group
4.	1573cm^{-1}	C=C Stretching	Aromatic Ring
5.	1407 cm^{-1}	C=N Stretching	Heteroaromatic Compound(In Ring)

Table 10: Determination of Functional Group by IR Spectra

Absorption Band	AH	DAA	DAC	DAS	KS
CH Stretching	✓	✓	✓	✓	✓
NH Stretching	✓	✓	✓	✓	✓
C=O Stretching	✓	✗	✓	✗	✓
C=N Stretching	✓	✓	✓	✓	✓
OH Stretching	✓	✓	✓	✓	✗
NH_2 Stretching	✗	✗	✗	✗	✗
NO_2 Stretching	✗	✗	✓	✗	✗

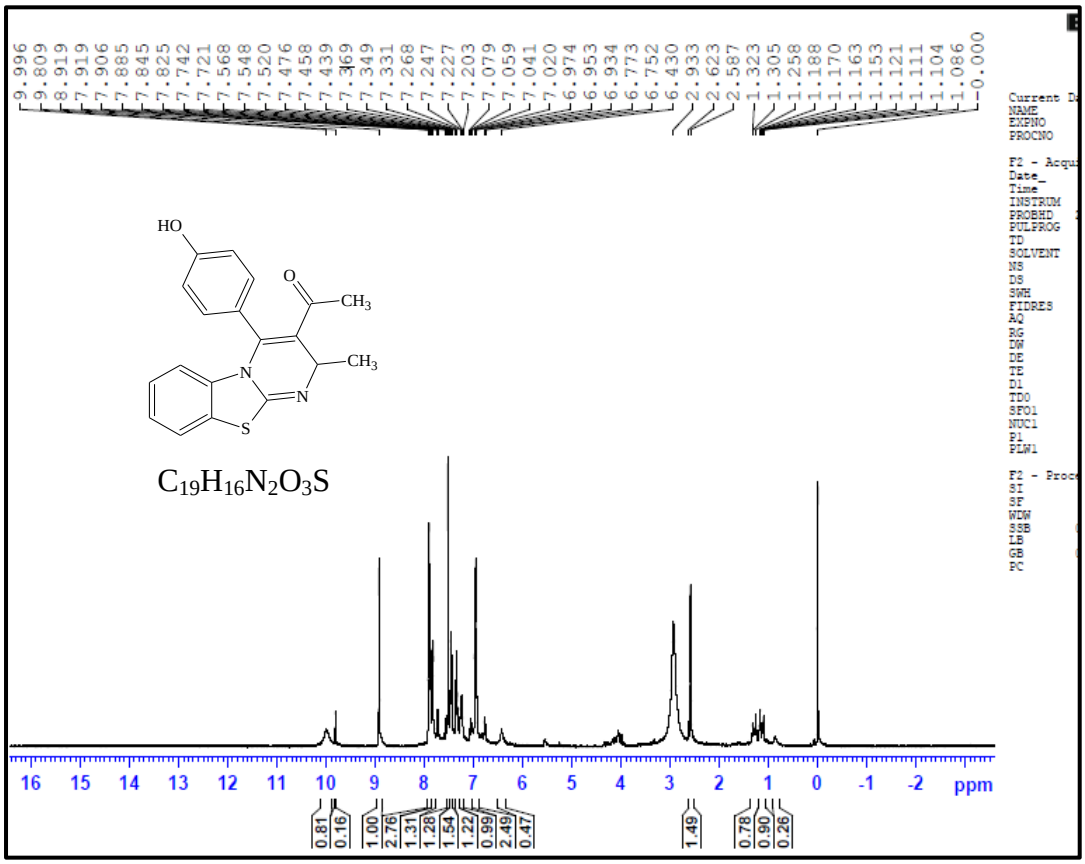
The IR spectrum will be used to identify the functional group of the synthesized compound. It also shows the absence of the functional group peaks of the parent compounds and the presence of the new functional group peaks of the synthesized compounds.

The presence of new functional group region:

C=N stretching in the region of 1300-1600cm^{-1}

NMR SPECTROSCOPY

Sample Code: AH

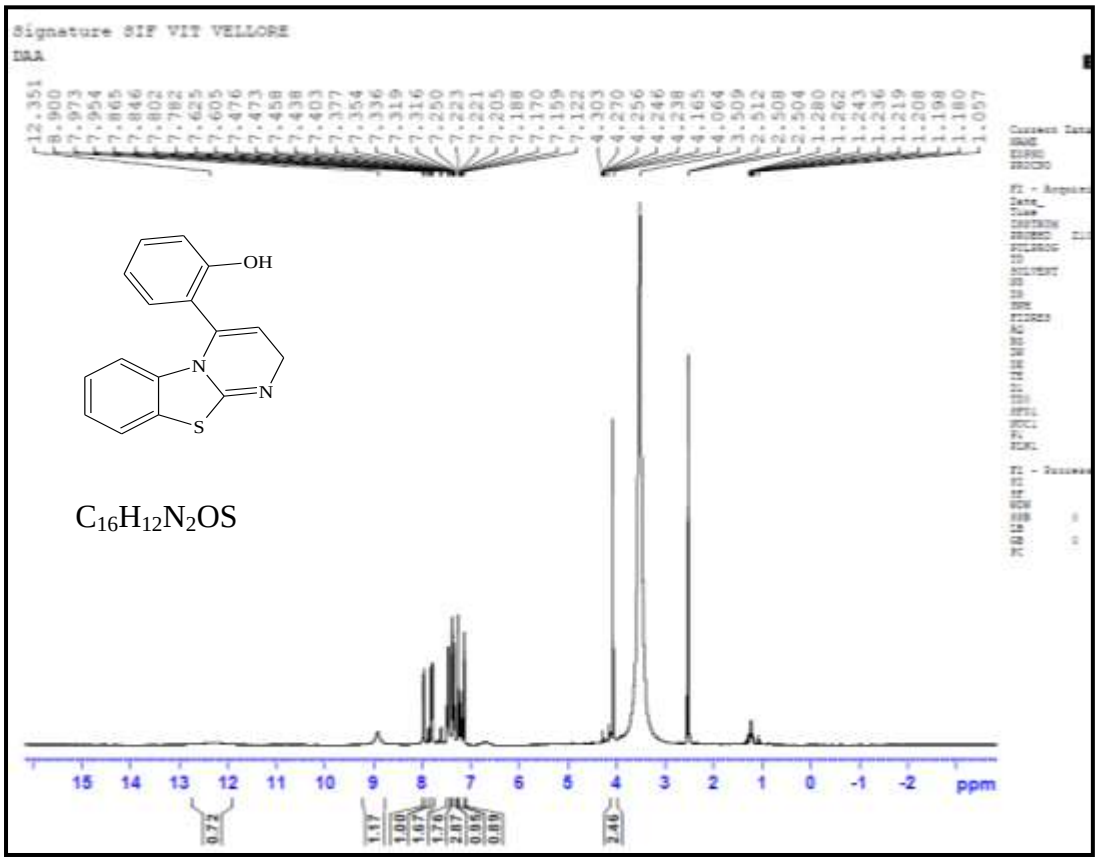


^{1}H NMR Spectrum of AH

Table 11: ^{1}H NMR Interpretation of AH

S.NO	δ VALUE	TYPES OF PEAK	NUMBER OF PROTONS
1.	9.9-10.1	Singlet	1
2.	6.7-8	Multiplet	4
3.	2.9	Singlet	1
4.	2.5-2.6	Doublet	2
5.	1.1-1.3	Multiplet	4

Sample Code : DAA

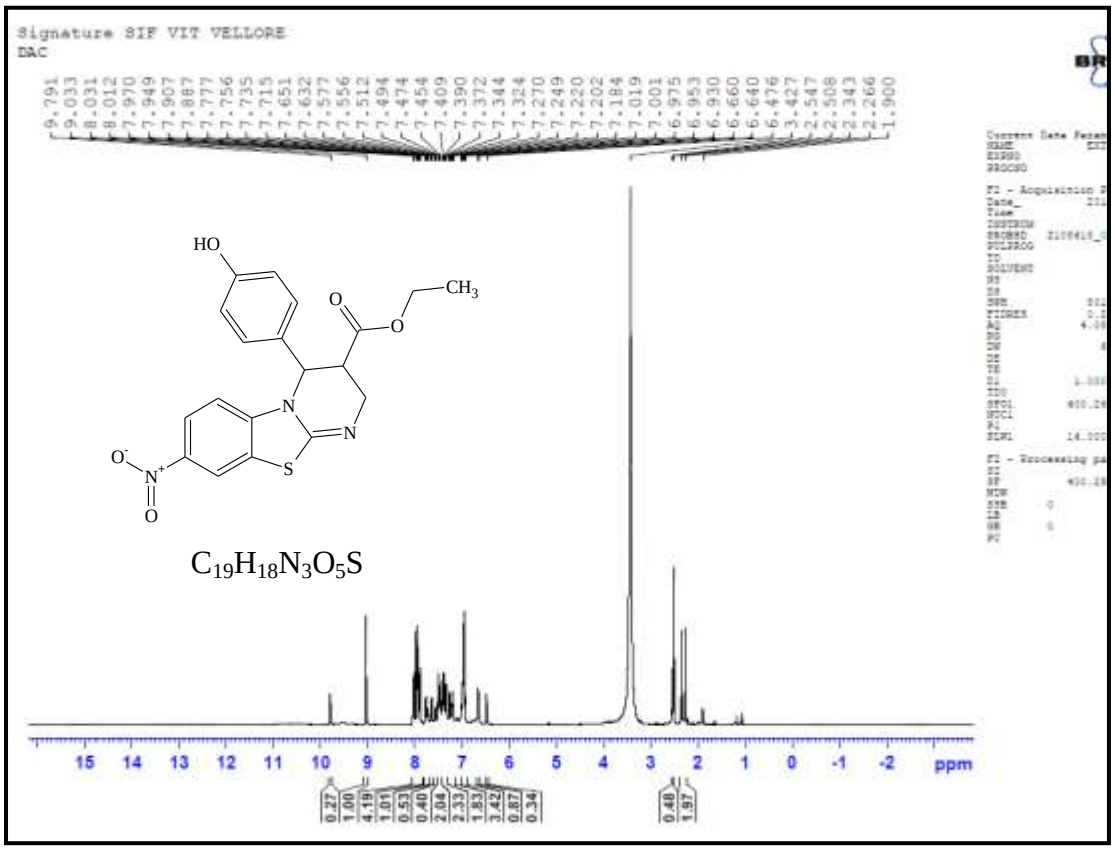


^{1}H NMR Spectrum of DAA

Table 12: ^{1}H NMR Interpretation of DAA

S.NO	δ VALUE	TYPES OF PEAK	NUMBER OF PROTONS
1.	7.9-8.0	Doublet	1
2.	7.7-7.8	Doublet	2
3.	7.1-7.5	Multiplet	7
4.	4.1	Singlet	2

Sample code : DAC

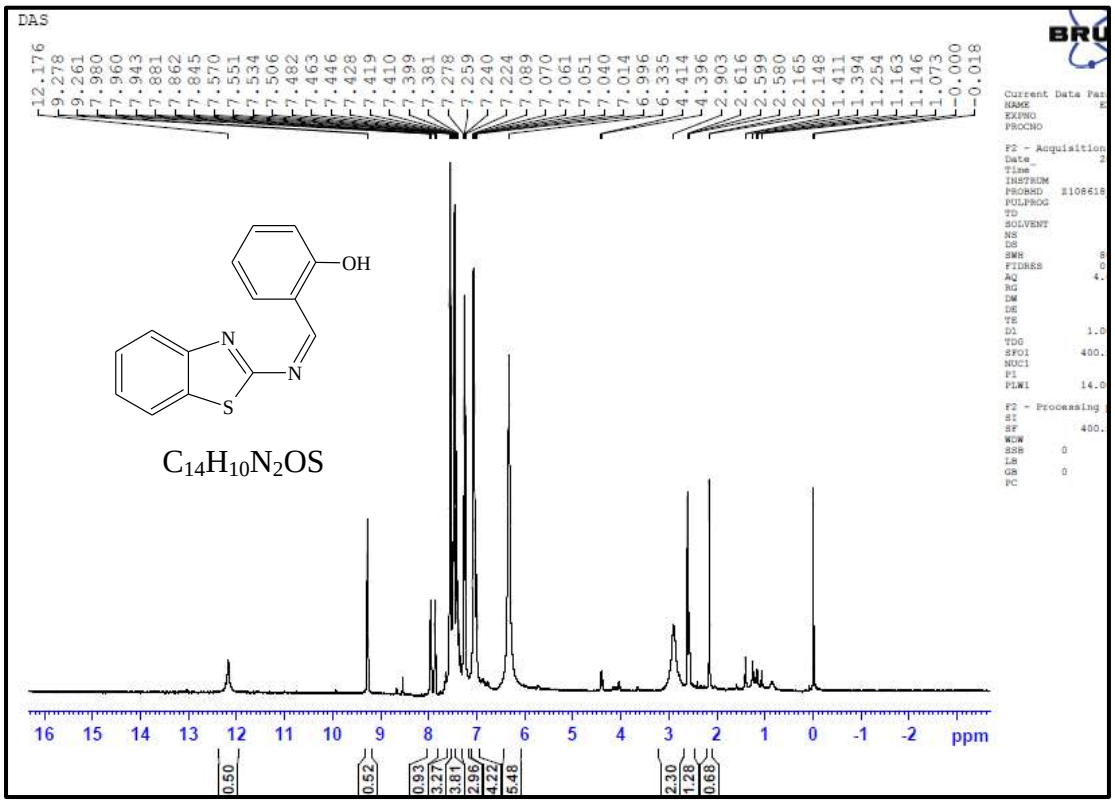


^{1}H NMR Spectrum of DAC

Table 13: ^{1}H NMR Interpretation of DAC

S.NO	δ VALUE	TYPES OF PEAK	NUMBER OF PROTONS
1.	7.9-8.1	Multiplet	4
2.	7.1-7.8	Multiplet	8
3.	6.9-7.0	Multiplet	3
4.	6.5	Singlet	1
5.	2.2-2.4	Doublet	2

Sample code : DAS



^{1}H NMR Spectrum of DAS

Table 14: ^{1}H NMR Interpretation of DAS

S.NO	δ VALUE	TYPES OF PEAK	NUMBER OF PROTONS
1.	12.1	Singlet	1
2.	9.2	Doublet	1
3.	7.8-8.0	Multiplet	2
4.	6.9-7.8	Multiplet	4
5.	6.2-6.4	Singlet	1
6.	2.5-2.6	Triplet	1

Sample code: KS

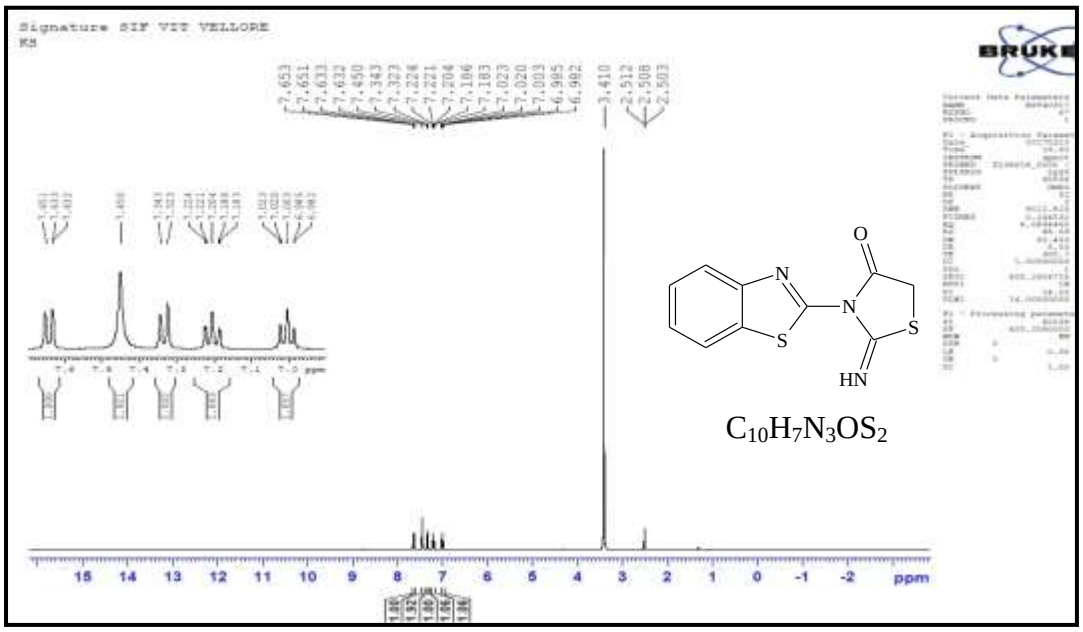


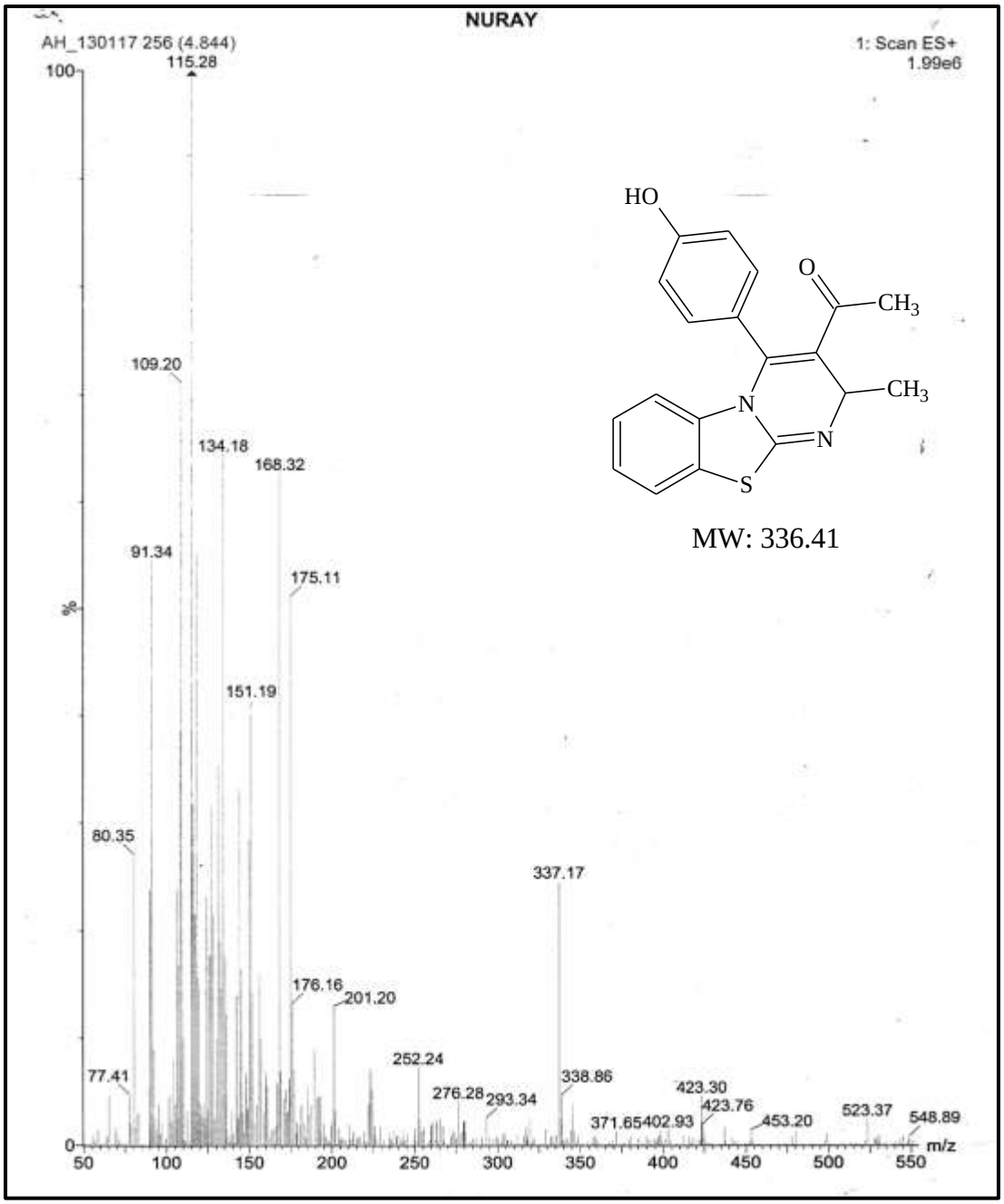
Table 15: ^{1}H NMR Interpretation of KS

S.NO	δ VALUE	TYPES OF PEAK	NUMBER OF PROTONS
1.	7.0-7.1	Multiplet	1
2.	7.1-7.3	Triplet	1
3.	7.3-7.4	Doublet	1
4.	7.4-7.5	Singlet	2
5.	7.6-7.7	Triplet	1

The ^{1}H NMR Spectral data of all the synthesized compounds were used to assign molecular structure. A short sharp singlet was observed at 4.5-6.5(ppm) for all compounds, which might be due to the presence of NH proton. All the compounds showed multiplet signals for the presence of aromatic protons between 6.4-8.0(ppm) and Heteroaromatic protons between 5.5-6.5(ppm). The singlet peak was observed at 8.5-12 (ppm) for AH, DAA, DAC and DAS compounds , due to the presence of OH proton.

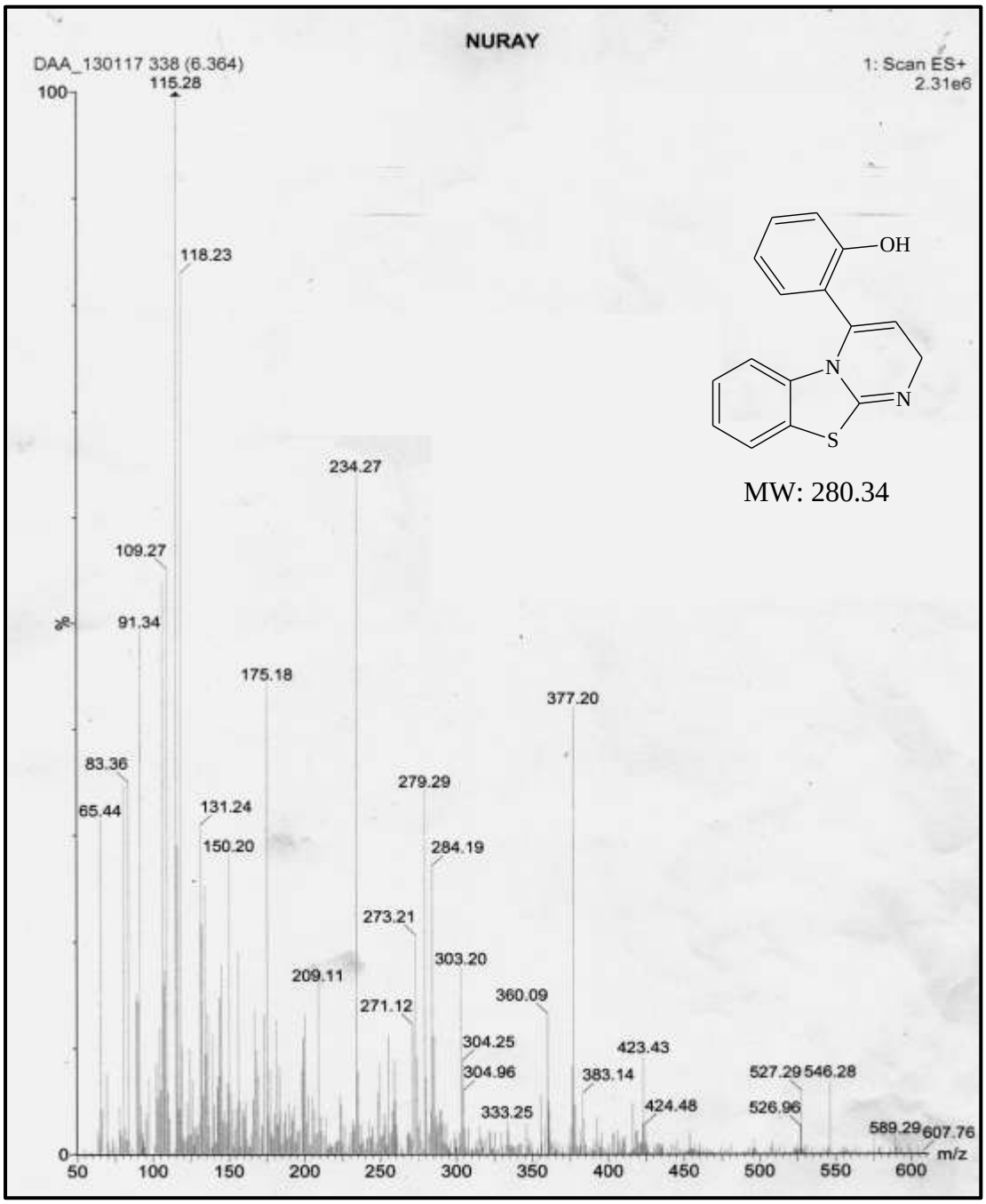
MASS SPECTROSCOPY

Sample Code: AH



LC –MS Spectrum of AH

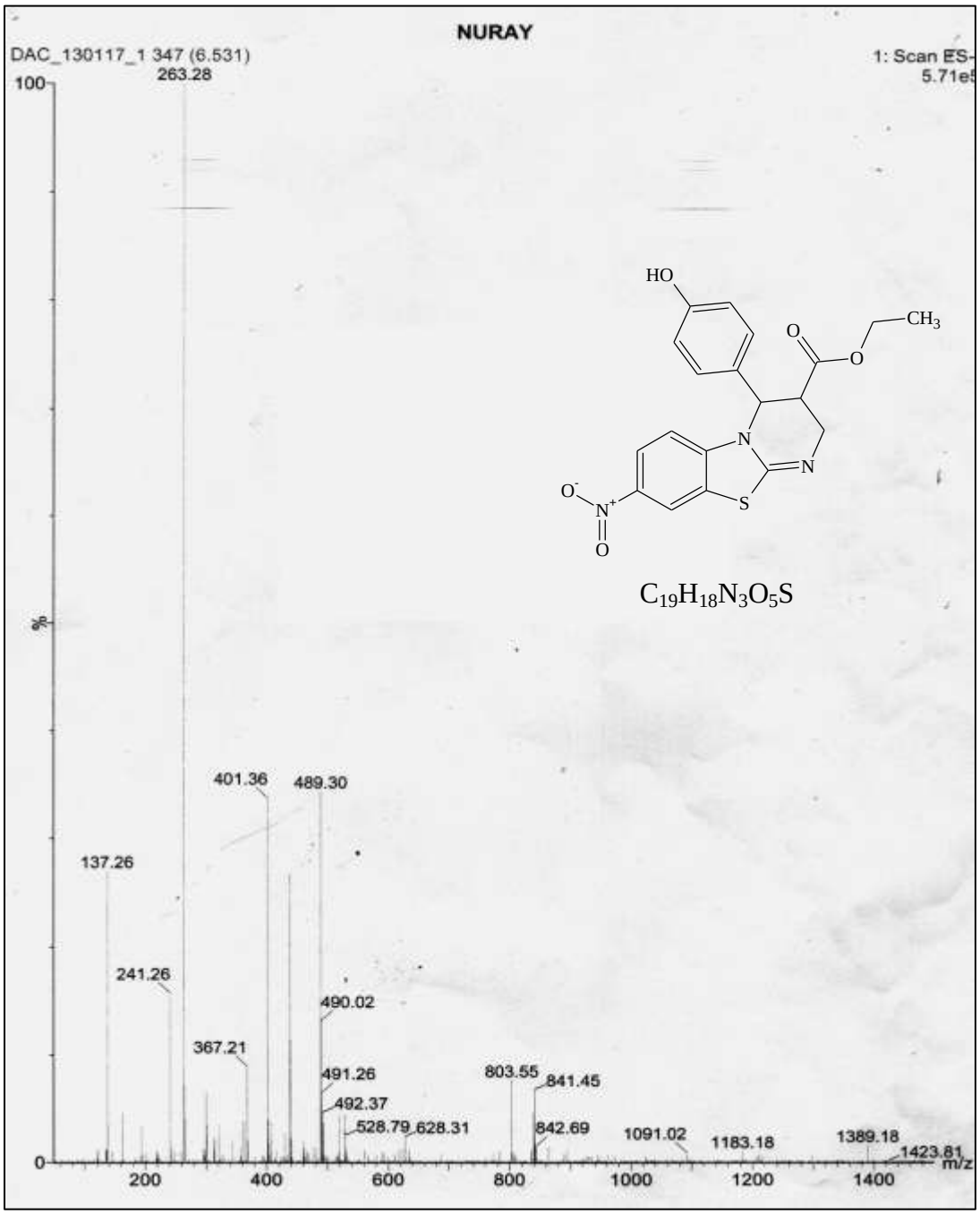
Sample Code: DAA



MW: 280.34

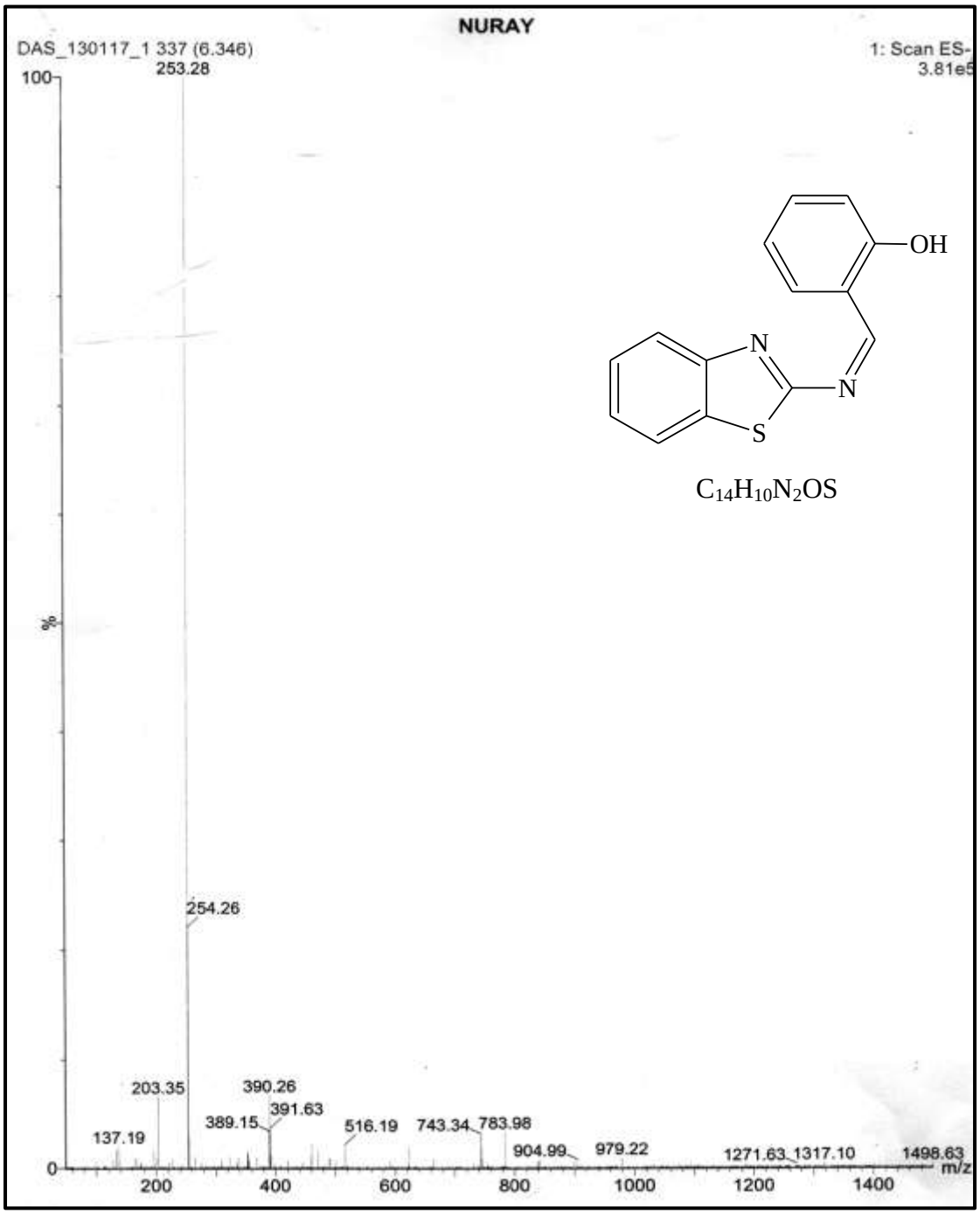
LC –MS Spectrum of DAA

Sample code: DAC



LC –MS Spectrum of DAC

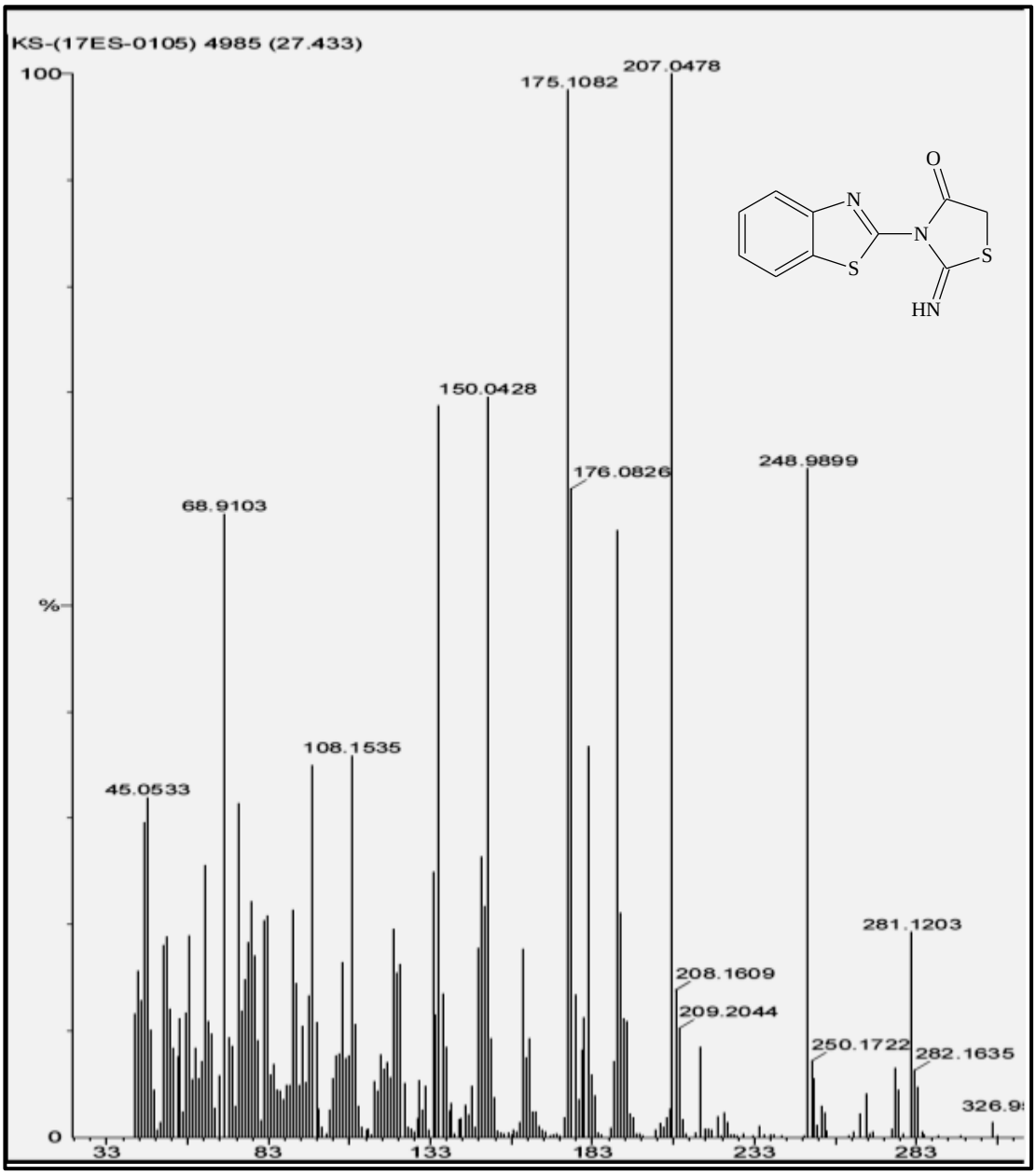
Sample code: DAS



$C_{14}H_{10}N_2OS$

LC –MS Spectrum of DAS

Sample code: KS

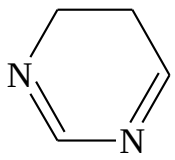


GC –MS Spectrum of KS

Table 16: Molecular Weight Determination by Mass Spectra

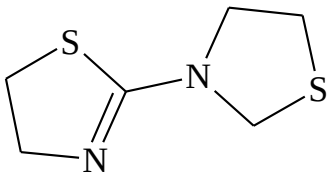
S.No	SAMPLE NAME	ACTUAL MASS	CALCULATED MASS
1.	AH	336.41	337.17
2.	DAA	280.34	279.29
3.	DAC	400.42	401.36
4.	DAS	254.31	254.26
5.	KS	249.31	249.15

All the synthesized compounds exhibited molecular ion peak (M±1) of varying intensities established the molecular weight of the compounds. It was observed that the compounds (AH, DAA, DAS, DAC) have fragment ions at m/z value of 83.36 and KS have fragment ions at m/z value of 175.1which probably due to the following structures.



4,5-dihydropyrimidine

m/z : 82.05



2-(1,3-thiazolidin-3-yl)-4,5-dihydro-1,3-thiazole

m/z :174.10

IN-VITRO ANTITUBERCULAR ACTIVITY

Compounds were screened for *invitro* anti tubercular activity (0.8-100µg/ml) by Microplate Alamar Blue Assay. The organism H37Rv used in the study i.e. Mycobacterium tuberculosis. The anti mycobacterial activity of the compounds was assessed against M.tuberculosis using Microplate Alamar Blue Assay (MABA).

- All the synthesized compounds showed anti-mycobacterial activity in varying degrees against the organism tested.
- The organism tested was susceptible to all the synthesized compounds and the minimum inhibitory concentration for the compounds varied between 12.5 and 6.25µg/ml.
- The data pertaining to these observations are presented in the table.
- Inhibition was compared using Pyrazinamide- 3.125 µg/ml, Ciprofloxacin-6.25µg/ml and streptomycin- 6.25µg/ml as standard.

Table 17: Biological Evaluation of the Synthesized Compounds

S.No	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1.	AH	S	S	S	R	R	R	R	R
2.	DAA	S	S	S	S	R	R	R	R
3.	DAC	S	S	S	S	S	R	R	R
4.	DAS	S	S	S	S	R	R	R	R
5.	KS	S	S	S	S	R	R	R	R

R – RESISTANT; S – SENSITIVE

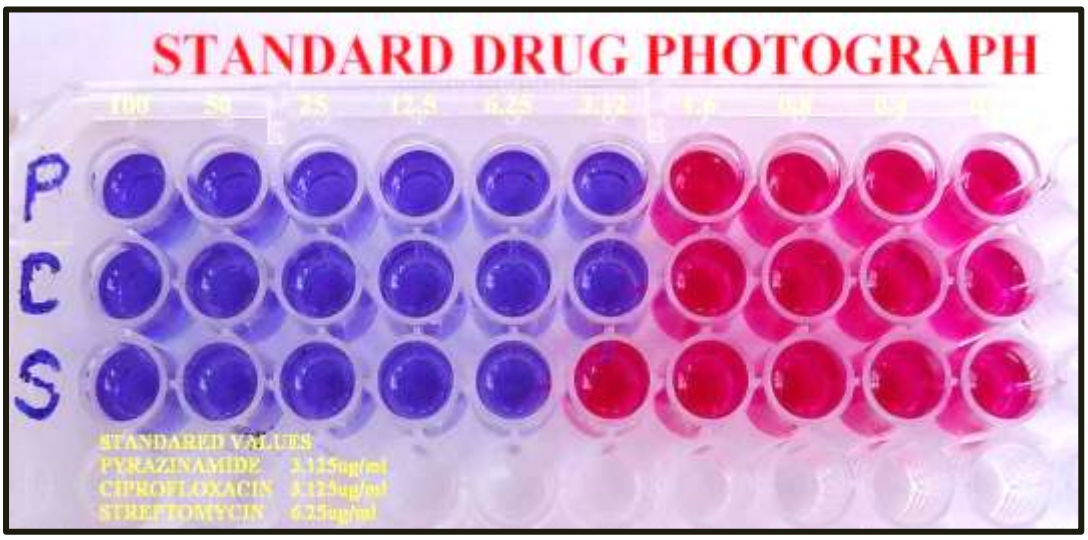


Figure 23: Standard Drug Photograph

Strain used: **M.tuberculosis** (H37RV strain): ATCC No- 27294

Pyrazinamide – 3.125µg/ml; Streptomycin – 6.25 µg/ml;

Ciprofloxacin – 3.125 µg/ml

NAME	100 µg/ml	50 µg/ml	25 µg/ml	12.5µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
DAA								
AH								
DAS								
DAC								
KS								
DNS								

Figure 24: Biological Evaluation of Synthesized Compounds

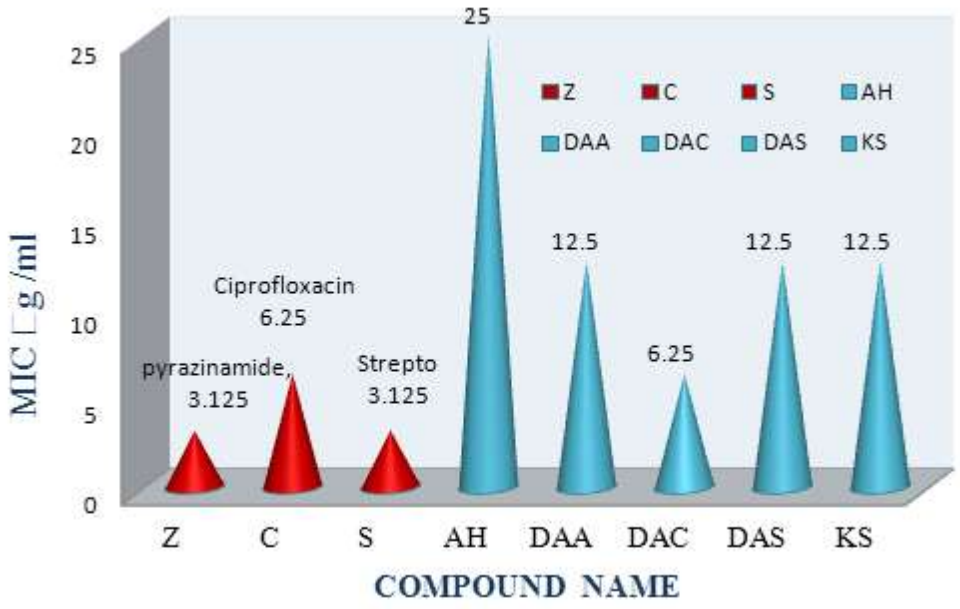


Figure 25 : MABA assay

COMPARATIVE STUDY OF SYNTHESIZIED COMPOUNDS WITH FIRST LINE ANTI-TB DRUG

Table 18: Comparative Study with Standard Drug

NAME	MOLECULAR WEIGHT g/mol	DOCKING SCORE Kcal/mol	MABA ASSAY (MIC) µg/ml
PYRAZINAMIDE(Z)	123.11	-4.41	3.125
AH	336.41	-7.51	25
DAA	280.34	-7.77	12.5
DAC	400.42	-8.45	6.25
DAS	254.31	-6.67	12.5
KS	249.31	-6.41	12.5

The biological evaluation of the compounds found that they were sensitive at 12.5-6.25µg /ml to the specific organism and shows less activity to the standard drugs. The synthesized compounds have the better docking score than Pyrazinamide. This is the way indicates the limitation of docking software.

Table 19: Molecules docked against different targets

Name of the Enzyme	**Docking Scores Kcal/mol**				
	AH	DAA	DAC	DAS	KS
Glutamine Synthetase 1	**-7.5**	**-7.7**	**-8.4**	**-6.6**	**-6.4**
Decaprenylphosporyl β-d-Ribose 2-Epimerase	**-6.8**	**-7.6**	**-6.6**	**-7.6**	**-5.5**
Cyclopropane Mycolic Acid Synthase 2	**-7.0**	**-5.9**	**-6.5**	**-7.9**	**-4.4**
Methoxy Mycolic acid Synthetase 2	**-5.1**	**-6.4**	**-7.3**	**-6.8**	**-7.7**

It is concluded that the synthesized compounds have best docking score around 6.5-8.5 Kcal/mol against Glutamine synthetase 1, which is more important in the cell wall synthesis of the Mycobacterium tuberculosis. Also they have related docking score against various target. sTherefore, the compounds are capable of inhibiting Decaprenylphosporyl β-d-Ribose 2-Epimerase, Cyclopropane Mycolic Acid Synthase 2 and Methoxy Mycolic acid Synthetase 2.

SUMMARY

- Glutamine synthetase 1, critical enzyme for the cell wall synthesis of Mycobacterium tuberculosis was chosen after the review of literature.
- A database of 100 molecules with high probability of inhibiting the target Glutamine Synthetase was chosen by making changes to a known inhibitor scaffold that is aryl substituted benzothiazole nucleus was chosen for the study.
- In silico drug likeness properties were determined by using Molinspiration® Cheminformatics software based on the Lipinski's Rule of Five.
- Docking of the 3D structures of these 100 entities against the 3D structure of Glutamine Synthetase gave an insight about the energetic (molecular docking) by using AUTODOCK® 4.2.5.1 software.
- Of these 100 structures, only 5 structures which showed minimum binding energy were chosen for synthesis (around -6.6 to -8.0 kcal/mol).
- They exhibited the better docking score than the standard Anti-TB drugs like Pyrazinamide -4.41kcal/mol by using AUTODOCK® Software.
- Toxicity risk assessment prediction was done for all the 5 compounds by OSIRIS® property explorer which is available online. The results are colour coded as green colour, which predict the drug likeness.
- The purity of the synthesized compounds were confirmed by TLC and melting point and then characterized by IR, ^{1}H NMR, GC-MS and LC-MS.
- The pure compounds were screened for in vitro Antitubercular activity by Microplate Alamar Blue Assay method.
- The synthesized compounds showed sensitivity (Minimum Inhibitory Concentration) in the range 12.5-6.25μg/ml. The standard drugs

Pyrazinamide, Ciprofloxacin and Streptomycin exhibited antimycobacterial activity at 3.125µg/ml, 3.125µg/ml and 6.25µg/ml concentration respectively. This indicates that the synthesized compounds are as active as the standard drugs.

- ♣ The synthesized compounds showed better docking score than Pyrazinamide. However, all of them are less active than Pyrazinamide.
- ♣ The synthesized compounds have related docking score around 6.5-8.2 Kcal/mol against various target. Therefore, they are capable of inhibiting Decaprenylphosporyl β-d-Ribose 2-Epimerase, Cyclopropane Mycolic Acid Synthase 2 and Methoxy Mycolic acid Synthetase 2, which is also had the functional importance in the Mycobacterium tuberculosis.

CONCLUSION

It is concluded that the synthesized compounds might effectively inhibit the chosen target Glutamine Synthetase I which is essential for the Mycobacterial Tuberculosis. Further structural modifications of the synthesized compounds will aid in the development of potential molecule against the tuberculosis pathogen.

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